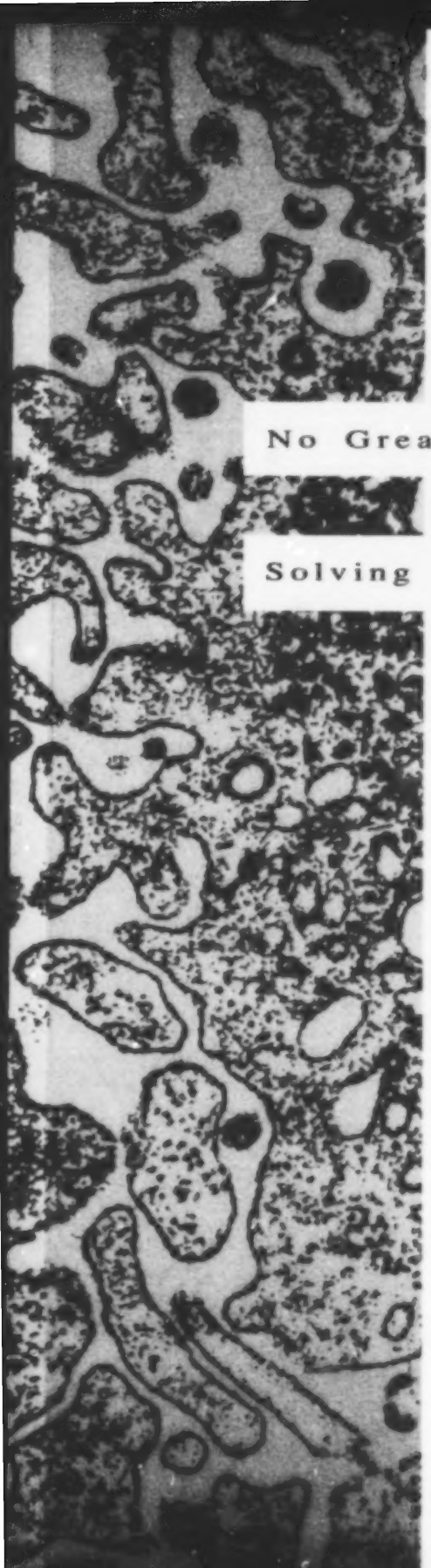
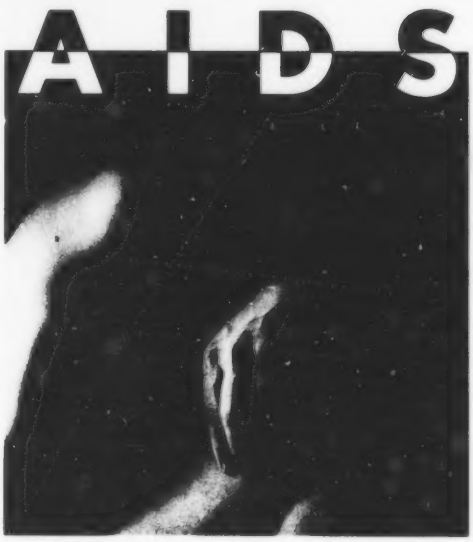


No Greater Challenge

Solving the Mysteries

AIDS



Medical Research Council of Canada
Conseil de recherches médicales du Canada

No Greater Challenge

Solving the Mysteries

AIDS

David Spurgeon

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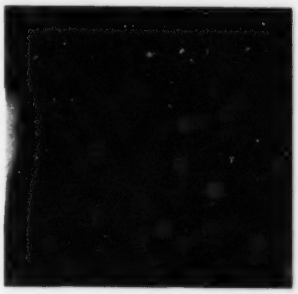


The acquired immunodeficiency syndrome (AIDS) constitutes an unprecedented global health challenge for all countries. Near the end of 1989, close to 200,000 cases had been officially reported in 152 countries. Many go unreported, however, and the

World Health Organization (WHO) estimated that by the end of the year the actual number would be closer to 600,000. The number of individuals infected with the causative agent, human immunodeficiency virus (HIV), is unknown, but WHO estimates it to be six to eight million. Tragically, most if not all of these will go on to develop AIDS.

Canadians have not been left untouched by this pandemic. In fact, among industrialized nations, with 3,130 AIDS cases in 1989 we ranked fourth highest per million population. The number of Canadians infected by HIV has been estimated at 30,000 to 50,000.

The Medical Research Council of Canada (MRC) considers HIV/AIDS research to be one of its major challenges. Its grantees include some who have been among the first to have been involved in such work anywhere in the world. As can be seen by the examples described in this publication, their approaches to the HIV/AIDS problem are bold, original, imaginative and productive.

Because of the enormity and the complexity of the challenge, such work must not only be continued but substantially expanded. The MRC hopes that this publication will help Canadians to understand how their medical scientists are responding to the challenge - and that it will encourage other researchers to join in.

Pierre Bois, President, MRC

What are Canadian researchers doing to help solve the serious public health problem posed by HIV infection and AIDS?

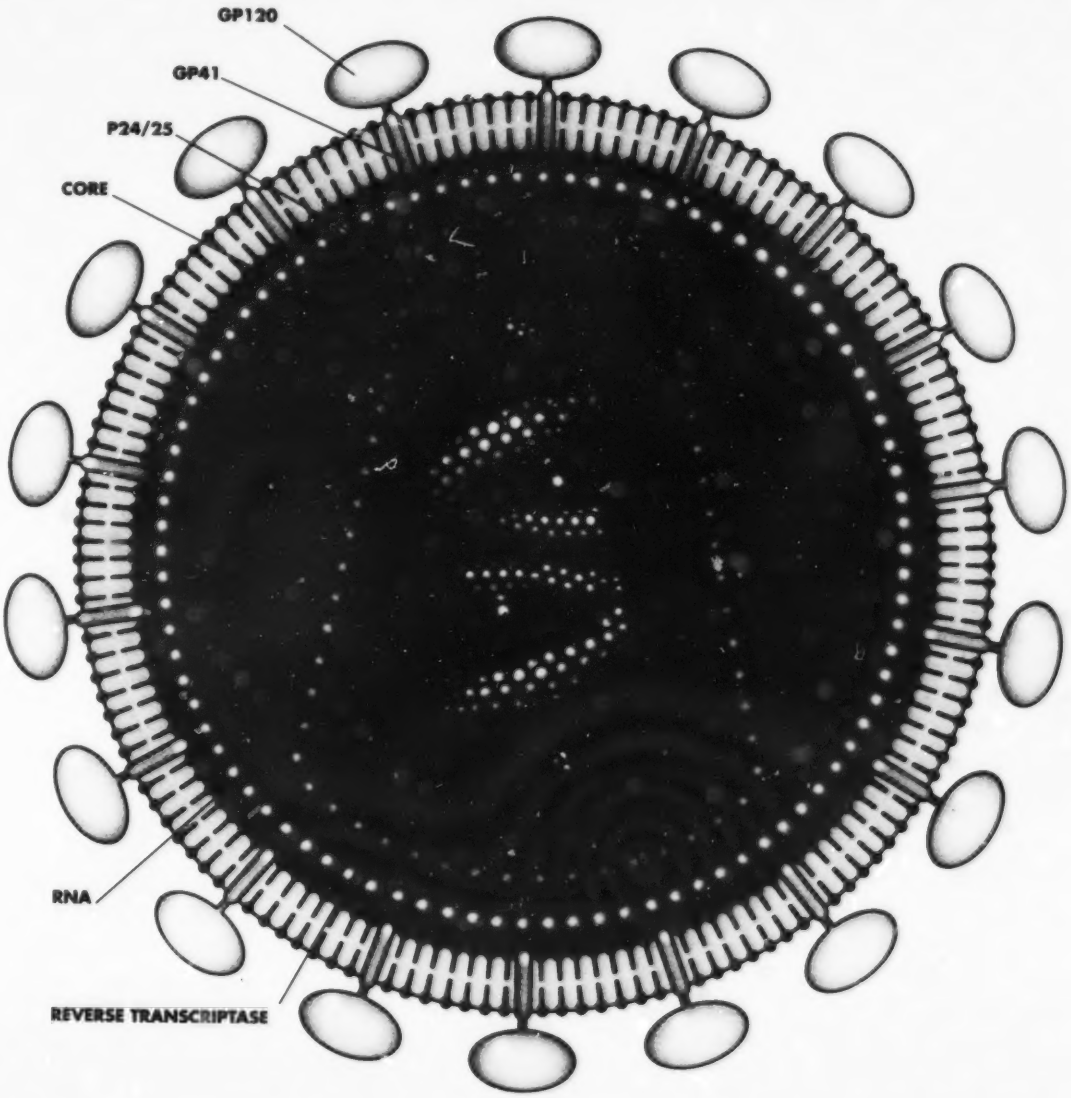
This booklet has been written in an attempt to explain some of the more important research projects being carried out now, in language comprehensible to readers with no specialized medical background.

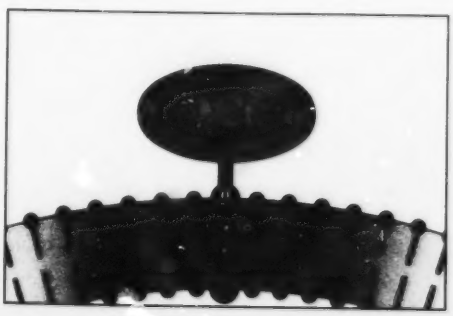
A technique employed in this publication is the journalistic interview. Although the lay person or the non-specialist might find it difficult to believe when reading scientific publications, many scientists can be remarkably articulate about their work when discussing it face to face. What also comes through is some of the drama involved, and the scientists' sense of excitement about what they are doing. There is, therefore, liberal use of direct quotes.

Part of the reason for this booklet was to encourage those who may be thinking about research as a career to consider the field of HIV, AIDS and retroviruses. For this reason those interviewed were asked how they became active in this particular field — and how in fact they began doing scientific research in the first place.

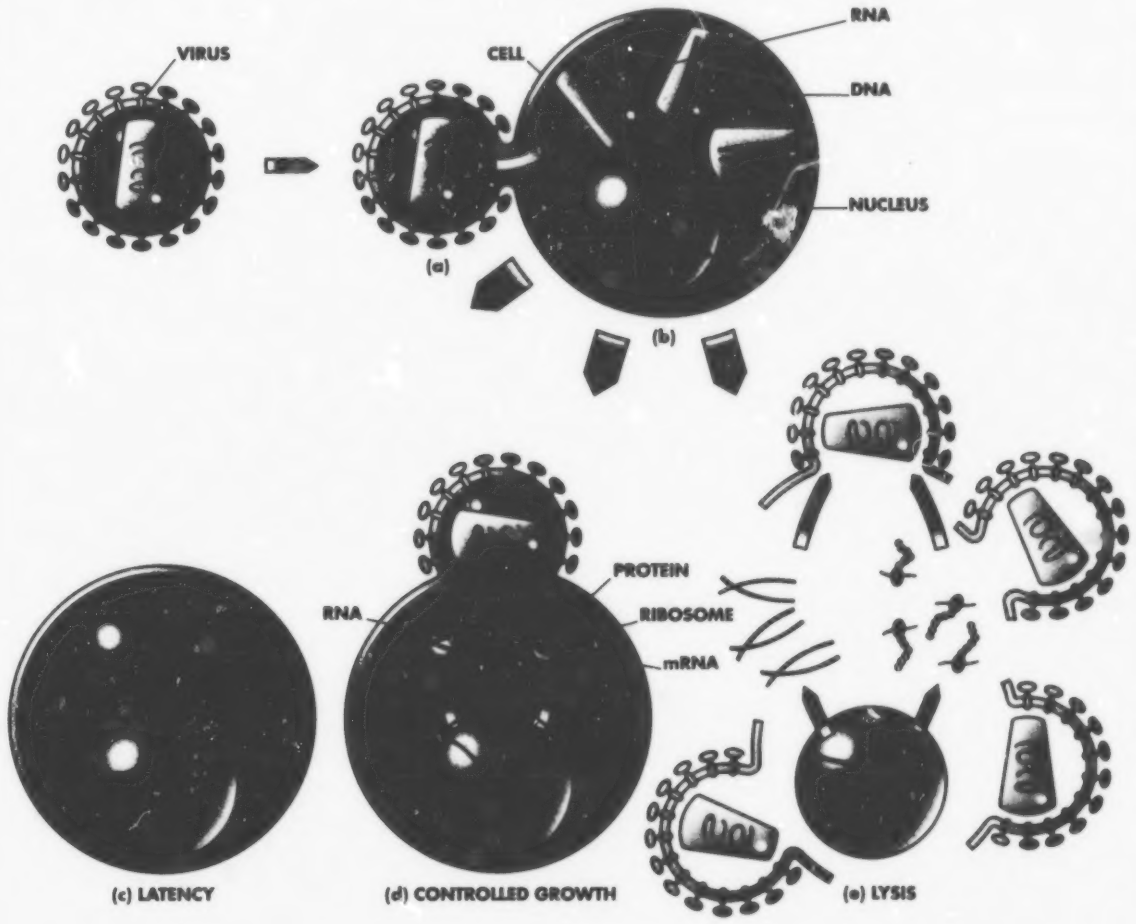
Another purpose of the publication was to learn how the work of Canadian AIDS researchers could be strengthened: what they lack, and how their work can be facilitated. Their answers to these questions are included.

In the following pages you will learn about the attempts of a number of Canadian researchers to clear up some of the remaining mysteries, with the goal of finding better treatment and control methods for HIV infection–and eventually if possible, a cure for AIDS.





HIV in cross-section: Knobs consist of gp120 protein, anchored by gp41 protein. String-like contents of knobs (in inset) represent the three protein molecules found in each knob. Virus core contains proteins gp24 or gp25; RNA, which carries the virus genetic information; and the enzyme reverse transcriptase. Enzyme enables virus to incorporate its RNA into cell's genes, which are DNA.



How HIV Infects: Virus binds to cell wall and injects its RNA inside (a). RNA is copied into DNA and becomes part of cell's own DNA within nucleus (b). After latent period (c), it may command cell to assemble new viruses that bud from cell (d). Viral reproduction may occur so rapidly that cell bursts, releasing viruses to infect other cells (e).

The picture thus presented must necessarily be partial and incomplete. Because of its complexity, AIDS research is extremely wide-ranging, and much basic research that could contribute to the understanding of AIDS could not be described here. This booklet is simply a series of snapshots of certain work being performed at a specific time, and is obviously based on current knowledge of HIV and AIDS.

Medical science has known about acquired immunodeficiency syndrome for less than a decade, yet most specialists in the field have agreed for some time upon its cause and the course of its development. The generally agreed description goes like this:

AIDS is caused by infection by the human immunodeficiency virus, which is carried in the blood and body fluids of infected persons. HIV can be passed from one individual to another via sexual intercourse, blood transfusion or contaminated hypodermic needles, or from mother to child during or around the time of childbirth.

HIV infection begins when the virus attaches itself, by means of a protein on its coat, to a molecule known as CD4 on the outside surface of a cell of the human immune system called a "helper T cell" (or T4 cell). Helper T cells are the "generals" that direct the battle of the body's defence forces against foreign invaders such as viruses and bacteria. They command the "B cells" to produce antibodies to neutralize the invaders, and "killer T cells" and "cytotoxic T cells" to destroy them. The helper T cells also control "suppressor T cells", whose function it is to "turn off" the immune response once an attack has been successfully repelled. (In HIV infections, unlike others, the antibodies produced by the immune system are unable to neutralize the virus, but their presence in the blood is a reliable indication that an HIV infection has taken place).

Once bound to the helper T cell, the virus invades it and incorporates its genetic information into that of the T cell. At this point the virus is dormant. The act of uniting with the body cell's genetic mechanism makes the virus invisible to the immune system. At some time in the future, responding to some as yet unknown signal, the invaded helper T cell starts reproducing new viruses. This destroys the T cell and frees new viruses to travel to other parts of the body. Sometimes these viruses are actually carried by the cells of the immune system whose job it is to engulf and eat invaders, known as macrophages[1].

Some cell types that do not carry the CD4 molecule can also be infected with HIV: one example is the "glial" cells that make up part of the nervous system. Once infected, these cells can produce new virus that can either infect other cells or lie dormant within them for some time. Infected glial cells are suspected as one cause of brain damage in AIDS cases, such as AIDS dementia. Macrophages can be infected whether or not they have CD4 molecules on their surface. Certain cells, such as the Langerhans' cells in the outer layer of the skin, and follicular dendritic cells in the lymph nodes, show limited amounts of CD4 and can be readily HIV-infected. Immature blood cells in the bone marrow (hematopoietic stem cells), fibroblasts and bowel epithelia can also be HIV-infected. Some of these may serve as reservoirs for the virus.

A person infected with HIV moves through a number of stages, the last of which is AIDS. A characteristic of HIV infection is that it may remain dormant for months or years: during this time infected persons may have no symptoms and show no signs of illness. Usually they feel perfectly well. For some, however, the first signs soon after infection may be similar to those of infectious mononucleosis: fatigue, fever, swollen lymph glands, and perhaps a skin rash. These may disappear, and subsequent stages may include chronically swollen lymph nodes (lymphadenopathy); the onset of opportunistic infections, which take advantage of the suppression of the immune system; symptoms such as fevers, night sweats, weight loss, and oral thrush (a yeast infection inside the mouth); severe or persistent viral or fungal infections of the skin and mucous membranes; and finally premature aging, deteriorating thought processes, personality changes and AIDS dementia.

While agreement is widespread about the main elements of this description, much remains uncertain about HIV infection and AIDS. Why, for example, does the time between HIV infection and development of AIDS vary so greatly — by months and even years — from individual to individual? And why do some individuals appear more susceptible than others to HIV infection? After almost a decade of experience, specialists in the field are not even certain that *all* who become HIV-infected will necessarily develop AIDS.

If AIDS is to be successfully controlled, it is vital to find answers to these questions and other equally important ones. In the following pages you will learn about the attempts of a number of Canadian researchers to clear up some of the remaining mysteries, with the goal of finding better treatment and control methods for HIV infection — and eventually if possible, a cure for AIDS.

Many other Canadian researchers are doing excellent work on AIDS. However, all whose work is dealt with in the pamphlet are receiving grants from the Medical Research Council of Canada. Many also receive funding from other sources; these are noted in each chapter.

David Spurgeon

[1] Macrophages are widely distributed and migrate through the body. They are found in connective tissue (where after injury they remove debris), and in lymph and in blood, from which they remove foreign particles.

DR. TAK MAK OF TORONTO USES VIRAL GENES TO HELP SOLVE HIV MYSTERIES.

Despite all that has been learned about AIDS in the few years since it was first identified, many mysteries remain. Even the details of some of the most basic disease processes remain unclear.

For example, exactly how does HIV kill the body cells it infects? Or how does the viral coat protein bind to the CD4 receptor on its target cell? If scientists fully understood these mechanisms, they might be able to block or disrupt them.

" That's how ideas come. You can wait all year long and nothing comes and then one day you sit down and it's there. I was in a meeting where people were discussing *tat*. They were going to try to do this and that, and I said to myself, 'Why don't they do *this*'? "

A Canadian researcher seeking answers to these problems is Dr. Tak W. Mak of the Ontario Cancer Institute (OCI). He's examining how HIV kills by first looking at what, in effect, turns the virus on. That may lead to ways of turning it off.

"We are trying to understand what can activate the virus", says Dr. Mak. "Inside it there is a protein called *tat* that is essential for the virus' expression. The idea is that if we can block *tat*, then the virus could not be expressed".

The Gene as *Impostor*

Tat is one of eight regulatory genes so far identified in HIV: its letters stand for *trans*-activator of transcription, and it is responsible for the burst of viral replication in HIV-infected T4 cells. The regulatory genes speed up or slow down the rate of viral protein synthesis. The others are *gag* (group-specific antigen), *pol* (polymerase), *env* (envelope), *rev* (regulator of virion protein expression), *vif* (virion infectivity factor), *vpu* (virion productivity factor *u*) and *nef* (negative regulatory factor).

"Fortunately, we have an opening", says Dr. Mak, "and that is that *tat* has very easy access to a cell: all you have to do is put it into a (growth) medium and it will find its way into the cell. So the idea we have is to synthesize a *tat* impostor that will go in and compete with the real *tat* in such a way that it will prevent the real *tat* from working, and the virus will not be able to express itself".

(One of the reasons *tat* homes so easily on target cells is that it carries a strong positive electrical charge while the cell surface is negatively charged. Exactly how it gets from there into the cell's nucleus is another mystery).

Dr. Mak's approach to the problem of how the virus binds to the CD4 molecule is based on roughly the same rationale he applies to the problem of how it kills cells: he is trying to synthesize reagents that can block absorption of the virus by the cell. To do this he produces in bacterial culture large quantities of the viral coat and receptor proteins, and then through repeated experiments tries to find chemicals that will block the binding.

"For the time being we are testing with so-called chemical libraries", says Mak. These are enormous collections of commercially-produced, coded chemicals that can be purchased and screened for possible blocking action, one after the other. Mak's group plans to run tests with at least 60,000 of them.

"Drug discoveries have resulted from two basic approaches, the rational and the random", says Mak. "Different drug companies have had different philosophies. We are using both approaches: the first is very logical, and the second random".

A Canadian example of a successful search for a useful compound by the screening process is vincristine, discovered by Dr. Robert Noble in an extract of the periwinkle plant. The drug has been valuable in treating a form of leukemia. Another example is one of the most effective drugs used to treat solid tumours — cis-platinum. On the other hand, the drug Ara-C, another leukemia treatment, resulted from a purely rational approach: a DNA analog, it blocks DNA synthesis in malignant cells.

In their attempts to find an agent that will stop *tat* from functioning, Mak and his associates construct a mutant cell that contains what he calls "a reporter system" to monitor the activity of *tat*. Mak's reporter is a herpes simplex virus gene, "plugged into" the HIV cell. The activity of this viral gene is entirely dependent upon *tat*: when *tat* is functioning, the herpes gene functions and an anti-herpes drug called Gancyclovir can be used to kill the mutant cell. When *tat* is not functioning, the herpes gene fails to function, and Gancyclovir will not kill the mutant cell.

During the screening process, potential blocking agents are added to the system one by one, and when one is added that competes with the herpes gene for *tat*, it will stop the gene from working and the mutant cell will survive a dose of Gancyclovir. Thus the researchers can tell if the agent they have added can block *tat*'s functioning by seeing whether or not the mutant cell can be killed by the anti-herpes drug.

"When the gene stops working", says Mak, "then you know you have found an antagonist."

Mak says the idea for the system just came to him one day out of the blue.

"That's how ideas come. You can wait all year long and nothing comes, and then one day you sit down and it's there. I was in a meeting where people were discussing *tat*. They were going to try to do this and that, and I said to myself, 'Why don't they do *this*'?"

Mak's approach to the virus/cell binding problem is even simpler, he says. As we learned in the introduction, HIV attaches itself to the CD4 molecule on its target cells by means of the gp120 protein on its coat. Mak's team therefore first "makes lots of gp120 and lots of CD4". They coat thousands of plates containing micro-wells with CD4, and then add the viral coat proteins, which have been radioactively labelled. Ordinarily the gp120 and the CD4 would bind and this would show up when the plates are examined by electrofluorescent techniques, as a result of the radioactive labelling. But if when potential blocking agents are added binding does not occur, then, says Mak, "you've got a lead (i.e., promising) compound".

Dr. Mak spent the first part of his career working on viruses, and during the past six or seven years switched to immunology. Because HIV is a virus that attacks the immune system, it seemed natural for him to turn to AIDS research.

"It's a very rare combination", he says. "Most people get into AIDS research if they are a virologist or a molecular biologist or an immunologist — and I'm all three of them".

He trained first as what he refers to as "a hard core virologist" at the University of Wisconsin and the University of Alberta, where he did both undergraduate and graduate work. In the late 1970s he joined the Ontario Cancer Institute in Toronto. Later his laboratory there turned to molecular biology and he returned to Wisconsin for further training in that field with Dr. Howard Temin, a Nobel Prize winner[2].

About that time he became interested in what has been called "the Holy Grail of immunology" — the T-cell receptor. In 1983 he and his colleagues gained international recognition for their discoveries of the nature of the T-cell and their success in cloning its gene. In 1988, Dr. Mak was named leader of a team of prominent North American scientists doing AIDS research. The team's project, of which his OCI work is a part, is funded by the U.S. National Institute of Allergy and Infectious Diseases, and includes researchers from Harvard University and the University of California. He is the first scientist outside the United States to win funding under this program. Other AIDS-related research he is carrying out is funded by the Medical Research Council of Canada.

Mak first wanted to be a chemical engineer, but he found engineering too regimented. He also considered going to medical school, but when working as a summer student in a laboratory in Wisconsin he was attracted to the variety and challenge of doing research.

It's a job at which he works six or seven 12-hour days a week. But Tak Mak still finds time to play tennis and listen to classical music.

[2] With Dr. David Baltimore and Dr. Renato Dulbecco, Dr. Temin won the Nobel Prize in medicine in 1975 for the discovery of reverse transcriptase, the enzyme produced by retroviruses that enables them to take over the genetic machinery of human and animal cells.

Is HIV *per se* the cause of AIDS?

The discoveries of Dr. Paul Jolicoeur suggest there is more to this story.

The many uncertainties that remain to be resolved about the infective process that leads to AIDS have prompted some investigators to question whether HIV is necessarily the *sole* pathogenic agent responsible. Recently, the findings of Dr. Paul Jolicoeur, of the Clinical Research Institute of Montreal, suggest that another disease agent could also be involved.

Dr. Jolicoeur's work has been done on mice. He has been studying a severe immunodeficiency disease caused in the mice by a strain of leukemia virus that is defective. A defective virus is one lacking the genetic components necessary for it to replicate. When such a virus appears in animals, there is always a "helper" virus present as well to enable the defective virus to replicate.

Dr. Jolicoeur says there are striking similarities between symptoms in the mouse immunodeficiency disease and those found in humans with AIDS: they include swollen lymph glands, spleen enlargement, suppression of immune functions and an excess of immunoglobulin in the blood. Like HIV, the defective virus that causes it is a retrovirus.

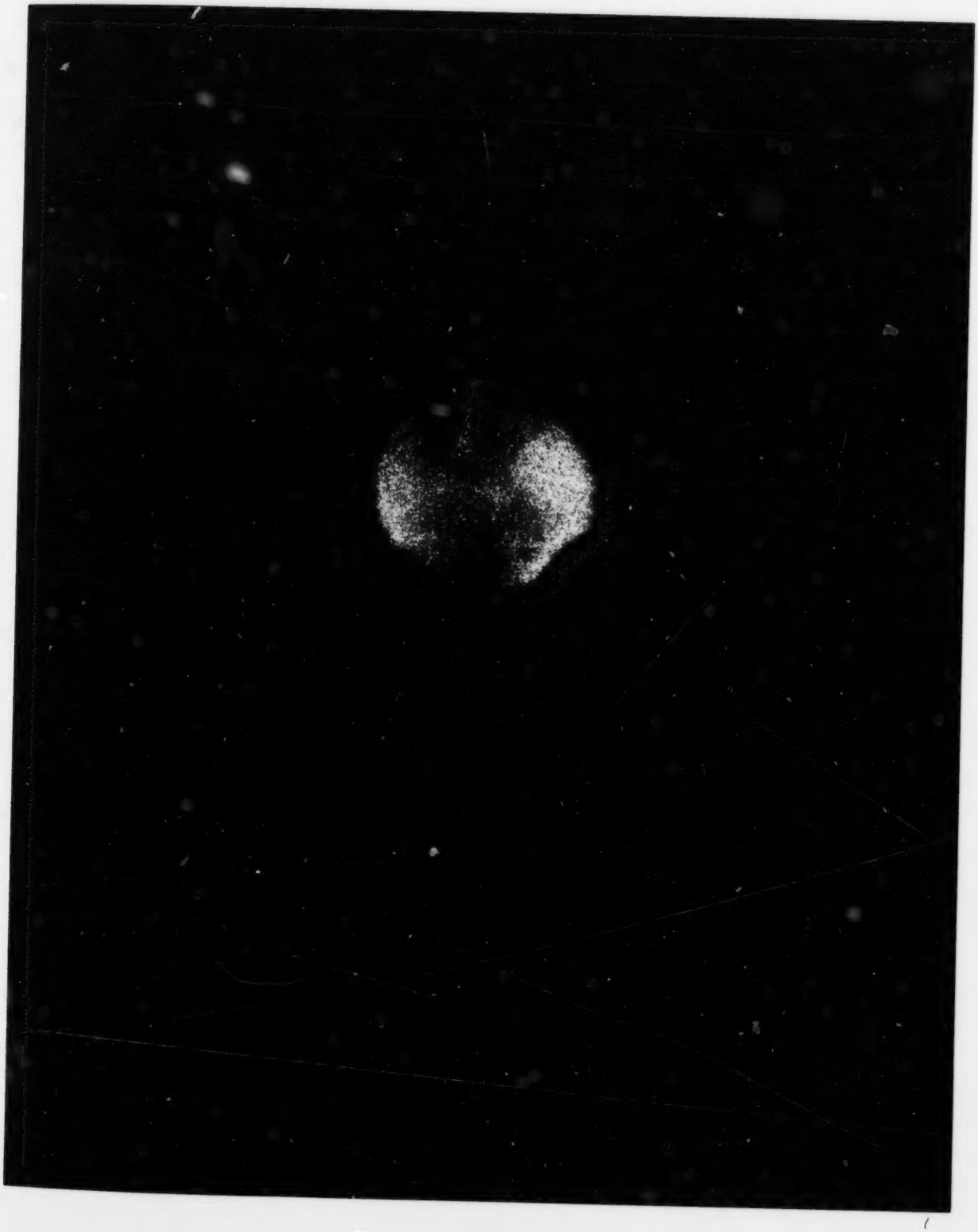
He and his colleagues have succeeded in isolating and cloning the defective virus, and when it was injected into other mice along with a helper virus that would not by itself cause the disease, it produced the disease symptoms within 8 to 12 weeks.

In an article published in April 1989, Dr. Douglas C. Aziz, now at Cytometrics Inc., San Diego, California, Dr. Zaher Hanna and Dr. Jolicoeur noted:

"This virus represents the second example, in addition to the variant defective FeLV (feline leukemia virus), of an immunodeficiency-inducing retrovirus that is replication-defective. Our results re-emphasize the need to search for similar pathogenic replication-defective variants in other immunodeficiency diseases, including AIDS, and suggest that the pathogenic AIDS virus may also be a defective virus".

If this turned out to be true, it would greatly change the way in which HIV infection is regarded. It would mean that *both* HIV and a defective virus would be necessary to cause AIDS. In this case, it would be the *defective* virus that would be the pathogenic agent (that is, the one that causes AIDS), not HIV, while HIV would be the *helper* virus. And if not all those infected with HIV also had the defective virus, only a fraction of those who showed positive results in an HIV test would be in danger of developing AIDS. Screening of the HIV-infected might reveal which individuals were in danger and which were not. Such a possibility is not just a flight of fancy: while blood tests reveal that the HIV antibody is present in virtually 100 per cent of people with AIDS, it may be that it

" The question is... are the different isolates of human HIV all pathogenic or not. We critically need to get this answered ".



is not only HIV — or HIV alone — that causes the AIDS. It is theoretically possible that the cause might be another disease agent in the blood that has not been detected.

"The question is", says Jolicoeur, " 'Are the different isolates of human HIV all pathogenic or not?' We critically need to get this answered".

Unfortunately, no suitable animal model has so far become available in which to test this hypothesis. Rhesus monkeys respond most like humans to retroviral infection and exposure to the simian virus, SIV, which is a sort of animal analog to HIV. But exposing them to HIV does not produce an immunodeficiency comparable to AIDS. Chimpanzees have been experimentally infected with HIV, but they have not developed AIDS. And because it is obviously out of the question to administer HIV to humans, the matter cannot be decided by direct experimentation. Thus, Jolicoeur's mice might turn out to be one of the best animal models yet. Mice are plentiful, inexpensive, and a great deal is known about their immune systems. In this case, the mouse disease closely mimics the human one.

"It's very common in animals to find defective viruses", says Jolicoeur. "All the defective viruses found so far cause cancer. They transform cells into cancerous cells. In fact that's how the whole field of cancer research has taken a new step recently — because of the discovery of oncogenes (specific genes that cause cancer). The first oncogenes to be discovered (in animals) were in defective retroviruses".

But in this case, the defective retrovirus does not contain an oncogene: it is only one of three such cases. According to Jolicoeur "the question that follows is: 'How does it cause the disease?' We are working very hard on that now". In addition to the research on mice, he and his associates are looking in the blood of human patients for defective viruses.

Jolicoeur made his discovery of the defective gene during attempts to clone the virus that caused the mouse immunodeficiency disease. However, at that time it was thought to be a cancer-causing virus, and he was interested in it for that reason. Describing how he examined the virus DNA using an electrophoretic technique called the Southern Blot, which shows its various segments in the form of bands on a gel, he said:

"When I put the virus on gel, I saw that it had an extra band — one that I had never seen before — and that was the defective band. We had been cloning many viruses, and we had never seen that, so I said 'if it's there, it must be important for the disease', and we went after it. It took a lot of biology to prove that it was indeed causing the disease. It could have been a totally unrelated thing. We just took a chance and cloned it. I was very excited when I saw that band on the gel".

At first they looked hard to see if the disease was triggered by an oncogene, as was the case with other defectives, but none was found.

Jolicoeur has great hopes for the significance of the finding. "I think it will lead to fundamental laws that will be applicable to any immunodeficiency. I think we're really getting at something important. You don't get an (animal) immunodeficiency that so closely resembles a human disease without a common background. For example, HIV could trigger the same pathway to produce immunodeficiency — maybe a different step in the pathway, but the same pathway".

Jolicoeur started doing retrovirology in 1973, and he got involved in AIDS-related work because "I almost felt obliged" to do so: "there aren't that many people trained in retrovirology". It was also, obviously "a very interesting problem". But in addition, "it's a social problem ... and I'd been working in Africa, and it's also an African problem". He was in Africa as a general practitioner in charge of a hospital in Togo in 1979 with CUSO (Canadian University Services Overseas). But what took him into medicine in the first place?

"I went into medicine for a single reason: "I wanted to become a psychiatrist", says Jolicoeur. "In fact I wanted to go into psychology, but some people convinced me I would have a better background if I became a psychiatrist, and that's why I decided to do an M.D. degree".

"Then, very quickly, I went into the laboratory. In fact in my first year of medical school a professor made it possible for me to do brain studies. During my electives I did almost a year of lab work".

At the end of his medical training, however, Jolicoeur decided on international medical work, and was accepted by the Harvard School of Public Health.

"One of the Harvard people asked me why I didn't go into the field to get a feel for it," he recalls. "So that's how I got into CUSO".

Jolicoeur's experience in Togo convinced him, however that Africans had more need of safe water and good sanitation than of medical practitioners. So he returned to take a Ph.D. in endocrinology with Dr. Fernand Labrie at Laval University.

" I went into medicine for a single reason: I wanted to become a psychiatrist. In fact I wanted to go into psychology, but some people convinced me I would have a better background if I became a psychiatrist, and that's why I decided to do an M.D. degree ".

He did postdoctoral studies at the Massachusetts Institute of Technology with Dr. David Baltimore, who with Dr. Howard Temin and Dr. Renato Dulbecco won the Nobel Prize in medicine in 1975 for the discovery of reverse transcriptase. Jolicoeur returned to Canada in 1976 as director of the laboratory in which he now works.

The current medical educational system, he believes, does not favor entry into research.

"There are many problems. One is that the training is long: the M.D. degree takes four or five years; then residency, two to three years; then Ph.D. and then a postdoc. Wow. That's hard, long and demanding. And you really have to do a postdoc if you want to compete. Many try to go into research three to four years after or during a residency, and when they come back they think they are researchers. They get wiped out very quickly by the system: it's too competitive and they don't have a good grasp of what science really is."

How Drs. Philip Gold and Chris Tsoukas made Canada a world centre for studies of AIDS and hemophilia and reduced the risk of HIV transmission through blood products.

Unknown to many Canadians, two Montreal medical scientists played a pivotal role in the early studies of HIV infection among hemophiliacs. Their work helped not only in the subsequent identification of the virus and the discovery of its role in causing AIDS, but also in developing methods to prevent the spread of HIV through the nation's blood supply.

Hemophiliacs at Risk

" The idea was to look at patients at very high risk of HIV infection. All hemophiliacs were getting their blood products from a serum pool that came from 50,000 to 100,000 people a year and they would all be at high risk ".

In 1982, there was only epidemiological evidence that AIDS was an infectious disease. The human immunodeficiency virus had not yet been identified and was not so named officially until four years later. The first cases of the disease were among homosexual men, and at that time it was known not as AIDS but as GRID, for Gay-Related Immunodeficiency Disease.

In Montreal that year, Dr. Phil Gold, now physician-in-chief of the Montreal General Hospital and chairman of McGill University's department of medicine, and Dr. Chris Tsoukas, currently assistant physician at the hospital and assistant professor of medicine in the university, heard of the first cases of a GRID-like syndrome among U.S. hemophiliacs. The reports alarmed them.

Dr. Gold was one of Canada's best-known immunologists and Dr. Tsoukas had done his residency and research in that field. Both were well aware of the risks hemophiliacs would run if such a syndrome were found to be caused by an infectious agent carried in the blood products that keep them alive. They also knew that complete records of Canadian hemophiliacs had been kept from birth, so they decided to press for a research project aimed at determining the connection — if any — between blood and blood products and the new immune disease.

"I had always had this tremendous desire to study a disease from the time before it began, through to the end", Dr. Gold recalls. "The idea was to look at patients at very high risk of (what later was identified as) HIV infection. All hemophiliacs were getting their blood products from a pool of serum that came from 50,000 to 100,000 people a year and they all would be at high risk".

Because the problem was urgent and applying for research grants can be a lengthy process, Drs. Gold and Tsoukas and others decided to appeal directly to medical research officials in Ottawa. With the help of the MRC, the project was immediately processed and they managed to obtain funds through the Special Projects Program of the MRC.

The result was the first and longest-running study of its kind anywhere, which has continued until this day. Dr. Tsoukas had kept blood samples from hemophiliacs from before the onset of the AIDS epidemic. These provided a unique record when HIV infection later began turning up among members of this group. Some of the samples became the first received by the U.S. laboratory of Dr. Robert Gallo, known as the co-discoverer of the AIDS virus.

"If you go to the U.S. Centers for Disease Control, they will tell you that their major resource in this entire area has been Chris' lab", says Dr. Gold. "It's the only lab that can give you the natural history of HIV-positive patients from before they get infected until death, including all of the phases of infection up to and including full-blown AIDS. It's an international resource".

Adds Dr. Tsoukas: "The key in getting it set up was the quick response by the MRC".

The situation of hemophiliacs has been tragic and could not have been foreseen. Many of them unwittingly found themselves among those infected with HIV and, subsequently, with AIDS. Their tragedy has, however, been of crucial importance in studying this disease.

The story begins when Chris Tsoukas was studying the immune system of people with hay fever. He was then a 34-year-old physician who had taken his residency in immunology at McGill. He'd got to that point by a highly circuitous route. And because it tells us quite a lot about the man who later became known as "the AIDS doctor" at a time when most physicians were avoiding AIDS patients, it's worth while to take the time to follow him along that route.

Chris came from a Greek family who lived in Montreal. After graduating from McGill in 1968 with a B.Sc. in microbiology, he wanted "to get away from home for a while". He chose Hawaii, because in his third year of university he had won a U.S. National Aeronautics and Space Administration scholarship that had taken him to the United States for a summer studying space biology with the astronauts under Carl Sagan. When NASA set up a centre in Hawaii, Chris went to a Ph.D. program in the University of Hawaii's microbiology department.

After receiving a master's in microbiology and immunology, he travelled to Asia and lived there for a year, doing relief work in the Mekong Delta during the Vietnam war. Later he spent three months teaching in a World Health Organization regional laboratory in Singapore.

Returning to Montreal, he did immunological research at the Royal Victoria Hospital, but decided he wanted to become a physician. He also decided to learn French, and after taking language courses went to Paris, where he enrolled in medical school. After some time, he went to Greece, his parents' homeland, where he accepted an offer of room and board from one of his aunts so that he could study medicine at the University of Athens. He graduated with his medical degree in 1977, and also interned there

When he came back to Canada, his plan was to return to Greece to live and practise medicine there. "But I met my future wife. I got married, and now I have four kids and I'm not going anywhere!"

Chris Tsoukas' research at McGill and later in Singapore had involved mostly "bench work", and he wanted more contact with people. It was this desire that got him into medicine, together with a familiarity with a doctor's life through observing his brother, who is a staff endocrinologist at the Montreal General Hospital. But when he did his residency, he realized that he would "like to use my brains more than my hands". And in the last six months of his internal medicine training he found McGill's immunology department exciting and his colleagues congenial.

Clinical immunology was particularly fascinating, at a time when "everything today seems to be immunologically mediated". During that period, hay fever was known as a "hot topic", involving as it did such advanced areas as T cells and monoclonal antibodies.

"One day I was in the lab at the cancer centre at McGill, when one of the residents came up to me and said: 'We have a hemophiliac in the hospital who has a telex from the CDC (the U.S. Centers for Disease Control) saying that hemophiliacs may get AIDS'", says Chris. The date, he remembers, was August 8, 1982. The hemophiliac was the president of the World Federation of Hemophiliacs, and thus had access to medical information long before it was generally known.

Tsoukas did some blood tests on this patient and found abnormalities in his immune system. The idea that these might have been caused by something transmitted through the blood products he was receiving for his hemophilia occurred to Tsoukas during a coffee break during a conference he was attending in New York.

"I got back here and talked to Dr. Gold and Dr. (Joseph) Shuster, all excited, saying I'd like to study the hemophiliacs. They were both extremely supportive, but the patient was even more helpful. He called up the company that made the monoclonal reagents (used in a blood test) and said he was a stockholder, and that he would like the company to provide me with samples of the reagents so that we could do a small project. Lo and behold a box arrived with about $5,000 worth of reagents".

Tsoukas then enlisted the help of the hemophilia centre in Montreal to recruit 34 hemophiliacs for the study. His idea was that in such a group, who received large amounts of blood products regularly but were otherwise healthy, abnormalities should show up if indeed they were caused by reagents.

"What was fascinating was that two thirds of the individuals had abnormalities", he said.

As a result of his findings, in January 1983, a meeting of Canadian and U.S. Red Cross and blood product manufacturing officials was held in New York City, to which Tsoukas was invited. "Behind closed doors and in a very profound and frightening kind of discussion, we discussed the possibility that the hemophiliacs were all going to get infected through blood products", Chris recalls. "We really had no marker (indicator) for AIDS and we could not understand the (test) results. The indications were that there was something wrong with their immune system".

A battle ensued for some time between those who believed the immune changes were secondary effects resulting from the presence of foreign proteins (i.e., the reagents), and those who believed they were the direct result of a virus.

"At that time, it wasn't clear", says Tsoukas. "It was very easy to take either side. At first I myself thought the blood factors were probably responsible for the immune changes".

Michael O'Shaughnessy, now with the Federal AIDS Centre in Ottawa, had good contacts with Gallo's laboratory in the U.S. He brought virus samples back to Canada and, wanting to develop ELISA and Western Blot tests and needing blood samples, he obtained those that Chris had kept from 1982. Between 1982 and 1984, the blood samples of 33 out of 34 of Tsoukas's hemophiliacs converted to HIV positive. And by this time, people outside the hemophiliac population who had received blood transfusions were coming down with AIDS.

Tsoukas recognized that more individuals were needed for a worthwhile study of hemophilia and AIDS, so he set about organizing a national effort. The MRC provided funds to buy a FACS (fluorescence-activated cell sorter) analyzer. This enabled collection of data on patients across the country.

"We then were able to follow the natural history of HIV infections and AIDS in hemophiliacs", says Tsoukas. "It became clear that there's no difference between the hemophiliac and the non-hemophiliac once they're infected with HIV. So whatever the blood products were doing, they weren't making any major changes to the clinical profile."

"What I also found was that you could follow people who were totally asymptomatic, felt well, and had no changes clinically over time, and yet in our lab test we could show that they had changing types of immune profiles. We did regression analyses and came up with some laboratory values that were useful in predicting AIDS progression".

Tsoukas' study is one of the largest anywhere and the longest-running. Whenever new knowledge about AIDS emerges from elsewhere, he can always pull his old blood samples from the freezer in which they are permanently kept, and derive new information from them.

The biggest contribution made by these Canadian studies has been towards safety of blood products. In 1983, the blood in banks throughout North America began to be subject to heat-treatment to make it safe from possible HIV contamination. But in 1987, some hemophiliacs who had used heat-treated blood factors began to show sero-conversion (positive HIV blood tests).

"We started a full investigation through the Federal AIDS Centre", says Tsoukas. "An epidemiologist went to Vancouver and tracked down a certain lot of a specific product from one company. Because I had all this information across the country we could localize it to one area. We could look at the records and see that no one else was sero-converting. And we could do things that could not have been done if a system like this had not been in place."

This led to a major change in heat treatment methods — from dry heat to the safer vapour treatment — of blood products worldwide. As a result, today hemophiliacs are treated with products that are not only safer from HIV infection, but also from infection from non-A, non-B hepatitis (a serious form so-called because it differs from both hepatitis A and B).

Finally, what Tsoukas considers probably the most significant accomplishment of the hemophilia study had nothing to do with AIDS: it was the finding that blood products, which are, after all, foreign proteins given over a hemophiliac's lifetime, produce no ill effects on their immune system. Says Tsoukas:

"A lot of people get human or animal proteins recurrently — diabetics get insulin injected every day, people who have pituitary dysfunction get pituitary extracts, those with allergies get allergy shots with pollens and animal danders, and so on. Although this is a fascinating issue, no one has really been able to analyze it properly. It took an enormous amount of data to be able to say with assurance that these proteins cause no immune system damage. Our body must have a certain resilience in handling the foreign proteins."

Drs. Gold and Tsoukas are emphatic about the lessons that need to be learned from this experience. Outstanding among them is the need for more manpower in AIDS research. In the beginning Tsoukas was working 12- to 18-hour days on the research and with AIDS patients, and looking, according to Gold, "more dead than alive". Recently the situation has improved somewhat, but is still basically the same after eight years.

Says Tsoukas: "In terms of AIDS-related basic research across the country, there is still great reluctance among investigators to get involved. You can count the investigators on one hand. On the clinical side the problems are numerous. We need commitments from provincial health authorities in co-operating with whatever program goes on nationally. The big thing we lack is a co-ordinated network of activities. We don't have enough patients for a proper clinical trial. You can't do it just in Montreal or Toronto — you have to go across the country. But there have been delays in setting up this kind of network and in getting clearances to work with one another. In terms of minor projects, there just aren't enough M.D.s looking after these patients. At individual hospitals, the problem has grown faster than their ability to look after it".

Underlying all these problems, Gold and Tsoukas agree, is lack of direction. The reason, says Tsoukas, is that "the people who should be there to guide us are not there. You need people at universities to push for research and we don't have them at many places".

Dr. Gold puts it even more strongly.

"What is needed", he says, "is that the federal government and the provinces identify AIDS as the major public health problem of our era".

" In terms of AIDS-related basic research across the country, there is still great reluctance among investigators to get involved. You can count the investigators on one hand. On the clinical side the problems are numerous. We need commitments from provincial health authorities in co-operating with whatever program goes on nationally. "

Drs. Chris Tsoukas and Philip Gold

The Search for a Better Way

DR. NIGEL PHILLIPS OF MONTREAL USES AZT-LOADED LIPOSOMES TO "TIME-BOMB" HIV-INFECTED CELLS.

"There has got to be a better way". That's what Dr. Nigel Phillips told himself as he thought about the serious side-effects suffered by HIV-infected people when they are given a drug meant to prolong their life. It's also what led him into a line of research that promises to produce a more effective and less toxic treatment.

A research associate at Montreal General Hospital, where he treks miles daily between his laboratory on the Côte des Neiges level and his tiny office five floors above the Cedar Avenue entrance, Phillips also is assistant professor at McGill University's department of medicine. A Briton, he received his doctorate from the University of London and postdoctoral training at Cambridge.

The drug he was thinking about was AZT (azidothymidine, now known as zidovudine), the anti-viral agent most widely-used in the treatment of patients with AIDS: it slows the otherwise inexorable progression of the acquired immunodeficiency syndrome. Since 1988, AZT has been used also experimentally to treat those who, while they test positive for the presence of HIV, have not yet shown symptoms: in such circumstances, it appears to stave off the development of AIDS.

But the trouble with AZT is that it is highly toxic: it has side-effects ranging from nausea, loss of appetite and insomnia to lung complications and severe toxic reactions with other drugs. More important, it homes in on the bone-marrow and interrupts the red blood cell manufacturing process, leading in some cases to anemia so severe that the treatment has to be stopped.

AZT works by slowing the replication of the virus. To do this it has to go where the virus is found in the body: in white blood cells called macrophages (residing in tissues, these literally eat invading viruses and bacteria but cannot eat HIV); in immature macrophages, called monocytes (which circulate in blood); and in other immune system cells called T cells.

Dr. Phillips' idea for a "better way" to deliver AZT to these targets was to devise a method of carrying it there, while at the same time preventing it from going to the bone-marrow, so that it could carry out its anti-viral activity without the toxic side-effects. The vehicle he chose is known as the liposome. He was well equipped for the job, because despite his youthful appearance (he's 40), Phillips has worked with liposomes as targetting agents for nearly 20 years.

" It's a bit like a time-bomb...we're putting a time-bomb of anti-viral agent inside a cell that is infected—or can be infected—with HIV ".

Liposomes are tiny, spherical sacs of water. When lipids (fat molecules) from body cells are placed in water, they form a membrane around it, creating a sac. Researchers have taken to placing drugs inside such liposome sacs, which carry them to difficult-to-reach sites in the body. Encapsulated in this way, the

drugs can be more effectively targetted to specific organs, and are less likely to produce unwanted side-effects through interaction with other bodily components en route. (Liposomes also have been employed for delivery within the body of enzymes an individual lacks, which is how Phillips started using them.) In addition, liposomes tend to avoid certain parts of the body, such as the heart, kidneys and gastro-intestinal tract. This lessens the uptake of drugs by these organs, and thus the drugs' toxic effects. Liposome drug delivery has been used frequently in cancer treatment, where the pharmaceuticals that attack cancer cells often harm other body cells as well.

In the case of AZT, it is the bone-marrow that must be avoided. Liposomes do not travel to bone-marrow, so when enclosed in liposomes, AZT's toxicity is greatly reduced. Where AZT is *wanted* is inside cells infected with HIV. To target these, Phillips decided to use what is known as monoclonal antibody techniques.

Antibodies are proteins the body produces to fight infections. As soon as an invading organism — such as a virus — enters the body, the body's immune system begins manufacturing antibodies designed specifically to seek out and neutralize the invader. Antibodies are highly specific: they fit the invader (the "antigen") precisely, the way a key fits a lock. In recent years, scientists have learned how to tailor-make antibodies in the laboratory, using genetic engineering techniques. This is done by "cloning" a specific antibody — producing identical copies in quantity. This "monoclonal antibody", when introduced into the body, then seeks out the specific antigen it was designed to fit, and neutralizes it.

Monoclonal antibodies are now available commercially, so Phillips purchased some designed to react with CD4, the molecule to which the human immunodeficiency virus attaches on the T cell's surface, and from which it invades and infects the cell. Phillips coats his liposomes with this CD4-seeking monoclonal antibody.

When they are injected into the body, the CD4-coated liposomes, carrying their cargo of AZT, naturally seek out macrophages, monocytes and T cells and enter them. Once inside the cell, the AZT goes to work, preventing the human immunodeficiency virus from replicating and thus slowing the spread of the infection.

"It's a bit like a time-bomb", says Phillips. "We're putting a time-bomb of anti-viral agent inside a cell that is infected — or can be infected — with HIV".

Near the end of the summer of 1989, Phillips' work was still being carried out in mice, but clinical trials among HIV-infected persons were already being planned in partnership with Dr. Chris Tsoukas.

"Chris is very interested in the clinical aspects and I'm very interested in the experimental approach, and it's something where we do have a lot of experience", says Phillips. "This wouldn't be the first clinical trial we'd have been involved in."

Phillips refers to the liposome work as "a marriage of a technique which has the potential for specific targetting with a disease area which almost cries out for specific targetting because of the toxicity of the anti-viral agents".

What is especially appealing about the method is its potential for long-term management of HIV infection. By continuously suppressing the spread of HIV within the bodies of those who are already infected yet are symptom-free, Phillips believes it could be used "in a prophylactic manner". The idea would be "to almost sterilize the lymphocyte population before a large number of cells have become infected", he says.

"I think of it as a treatment that would buy time for the development of vaccines. But its use should be early rather than late in HIV infection, in order to ensure that the proliferation of HIV is kept to the minimum, while at the same time saving the bone-marrow".

Clinicians already have started to use AZT in a prophylactic fashion with HIV-positive persons who have not yet shown AIDS symptoms, but they do not dare use large dosages because of bone-marrow toxicity. Phillips thinks liposome targeting "might result in a more effective therapy, because one would be able to use much higher doses of AZT without running into the problems of bone-marrow toxicity".

In previous work using liposomes for administering steroids to rheumatoid arthritis patients, for example, he found that one-twentieth to one-fortieth of the injection dose would have the same therapeutic effect when delivered by liposomes, without any side-effects.

Another useful attribute of liposome targetting is that the release of AZT can be controlled over a long period of time. Once the liposomes, loaded with AZT, enter the T cells, they begin to break down slowly, all the while releasing the drug.

"This is why I say they are like time-bombs", he says. "They are slow-delay devices. You can design the liposomes

so some break down immediately, some break down after 24 hours, some after 48 hours, some after 10 days. You simply change the composition of the liposomes. It's very, very easy. So you could have a series of liposomes which would break down at different rates".

With this sort of timed release, Phillips believes HIV infection could be controlled almost indefinitely, provided the AZT treatment was initiated early enough — "almost after diagnosis". If the HIV infection became too widespread within the body — for example entering the central nervous system — it might be too late, because liposomes do not cross what is called "the blood-brain barrier".

Such a treatment, he says, "might give a vaccine a chance to work". It's not currently known how long a vaccine might take to induce immunity to HIV, because no successful vaccines have yet been perfected. Yet for some tropical diseases, for example, it takes as long as six months.

"The ultimate goal is not only to target the anti-viral agent, but by suitable modification of the liposome, which is the vehicle, to actually control the antiviral agent's release so that it will produce a therapeutic concentration in the cells for a long period of time".

What motivated Nigel Phillips to take up such a complex research puzzle?

"Liposomes are extremely easy to make", he says. "And the materials that are used to make them are materials that you and I have in our own bodies. These are what are called phospholipids: every cell in our body is surrounded by a layer of phospholipids — it's what makes the cell membrane. So our bodies can handle it and degrade it and the potential for toxic by-products or contaminants is very small.

"But what switches me on in terms of using these things is that they have been shown to reduce the toxicity of a large number of drugs. My prime interest is in cells of the reticuloendothelial system — things like macrophages. And what's interesting about the liposome is that it naturally targets to the macrophage. So it's very easy to target a drug that affects the macrophage: if a virus can infect a macrophage, then the way to get the drug there is via the liposome.

"I've always felt that most of the anti-viral and anti-cancer treatments that we use require targetting to reduce their toxicity, because the toxicity limits their use. There are two approaches: one can search for derivatives or analogs that are less toxic, but which have the same anti-viral or anti-cancer effect. Or one can go the other way and say, 'Why not reduce the toxicity by specifically targetting the materials to where we want them to go?"

"The whole concept of trying to target things to the required site of action is really what motivates me. I find the whole area fascinating. The potential for increasing what's called the therapeutic index — the ratio of the beneficial effect to the toxic effect — by specific targetting is, I think, an approach which has been sadly neglected for the past 20 to 30 years".

But what led Nigel Phillips into science in the first place?

"I've been a scientist since I was 12 years old", he says. "My approach has been analytical since then. It wasn't a conscious decision, but when I started high school I entered into a science stream. You had the choice of either going into the arts stream or the science stream, and the prospect of doing Latin or French and everything else horrified me at the time".

That's no longer the case. Today he says he wishes he'd been able to do both — and in fact he is currently teaching himself Latin.

But as a youngster, Phillips was forever taking things apart and putting them together. "I had at least three chemistry sets a year, making smoke bombs and stink bombs and being a thorough nuisance to everybody, so there was no great conscious choice about coming into scientific research. I suppose, looking back, it's been almost a logical, natural progression: this is what interests me. I sometimes say if I had independent means and didn't need to earn a salary, I'd still do it".

Phillips recalls questions in job interviews that ask what his hobbies are. "The truth is", he says, "no scientist has a hobby. For a scientist, his work is his hobby".

Dr. Norman Camerman of Toronto examines molecular architecture in a search for ways of making more efficient anti-HIV agents.

Dr. Norman Camerman of the University of Toronto is half of what must be one of the most unusual collaborative teams in AIDS research: the other half is his identical twin brother, Dr. Arthur Camerman, at the University of Washington in Seattle.

" When AIDS came along, there was obviously a pressing need to develop anti-AIDS drugs. It seemed to us a natural thing to look at drugs that had shown some anti-HIV activity to see if we could come up with an explanation at the molecular level of how these drugs worked..."

Norman and Arthur took undergraduate and graduate degrees in chemistry together at the University of British Columbia. Norman now works in the University of Toronto's biochemistry department, while Arthur is in the University of Washington's departments of medicine and pharmacology.

Their collaborative research project itself is unusual, if not unique: it's concerned with the three-dimensional architecture of molecules that inhibit the growth of HIV. They hope the studies will reveal which stereochemical features of these molecules are responsible for their HIV-inhibition and why, thus enabling the Camermans to propose guidelines for improving these effects and for developing new anti-AIDS drugs.

"We're looking at agents that are useful against AIDS clinically, or that in pre-clinical tests have indicated some degree of efficacy in inhibiting HIV", says Norman.

Designer Drugs for AIDS

These agents fall into two main categories. One is a class of compounds he calls nucleoside analogs, which closely resemble components of the nucleic acids that make up the genetic material DNA. These analogs are believed to inhibit HIV growth in either (or both) of two ways: the first is by competing with natural nucleotides in binding to reverse transcriptase, the enzyme that allows the virus to incorporate its genetic material (RNA) into its target cell's DNA. By substituting itself for the natural nucleotide, the analog prevents the latter from binding to the enzyme and thus hinders the viral replication process. The other way is by resembling so closely one of the nucleotides that make up DNA — for example deoxythymidine — that the enzyme can't distinguish between the natural compound and the analog, and incorporates the analog into an aberrant DNA, thereby aborting the viral reproductive process. An example of this type of agent is AZT, the most commonly used anti-HIV drug.

The second category of compound is best exemplified by suramin, an organic dye developed in the 1920s as treatment and protection against trypanosomiasis (sleeping sickness).

"It was found a few years ago by a Belgian scientist named de Clercq that suramin was effective in inhibiting the growth of retroviruses", says Norman Camerman. "This was before the advent of AIDS, and when AIDS came along, Dr. Robert Gallo and his group, being aware of de Clercq's finding, tried suramin against HIV and found that, *in vitro*, it was extremely effective. It's been used clinically as well, and although it is virostatic (i.e., combats the virus), its side-effects are too toxic. It seems to suppress the immune system, which is exactly what you don't want".

So while suramin itself is not used as an anti-HIV treatment, less toxic analogs could be extremely useful.

The Camermans have determined the three-dimensional structure of suramin: it is not a nucleoside analog, and does not work in the same way as AZT. The method by which it inhibits HIV growth is believed to be by binding to what is known as the primer or template binding site of reverse transcriptase.

Reverse transcriptase requires this template — a strand of oligonucleotides — to which to bind in order to start its action. It also requires a primer — a short stretch of oligonucleotides — to which to add the nucleotides that will make up the viral DNA molecule.

"It's thought that suramin binds to this primer/template site of the reverse transcriptase", says Norman Camerman.

By growing crystals of suramin and then using X-ray diffraction, the Camermans determined the three-dimensional structure of suramin — a long and difficult task.

"We then used the following reasoning: if suramin acts by binding to the primer/template site of the reverse transcriptase, what is the molecule that normally occupies that site — what molecule is suramin imitating?"

Reasoning that the primer molecule would probably be a short stretch of either DNA or RNA, they then took one of the many published structures of oligonucleotides — which range from two to 12 nucleotides long and usually form helices — and compared it with suramin's structure by superimposing the two in three dimensions.

"We found, unexpectedly, that suramin is indeed a helical type molecule, and when superimposed with a six-nucleotide segment of DNA, there was an amazing resemblance both stereochemically and in the placement of electronegative groups between the two molecules. I think this enables us to explain at the molecular level why suramin will inhibit reverse transcriptase".

This was the first time the structure of suramin had been determined, and to Camerman's knowledge, the first time its anti-HIV action had been explained. The findings have been presented to a conference and a publication is being prepared.

"This is an extremely encouraging result because it enables us to try to make analogs of suramin that will more selectively bind to reverse transcriptase and weaken its binding to the enzymes that make DNA in normal cells", Norman Camerman says.

The reason for suramin's toxicity is its tendency to bind to enzymes of normal cells as well as to those of HIV: in effect, it attacks either indiscriminately. If drugs could be made that were suramin analogs yet were more selective in their action, their toxicity might be greatly reduced and their therapeutic usefulness greatly enhanced.

Recently, suramin has been shown to have anti-cancer properties, so the findings could have broader implications than for HIV alone.

Both the suramin and the AZT stories exemplify the Camermans' general philosophy.

"What we've been doing for some years is to try to get a rational understanding of the pharmacological action of drug molecules based on stereochemistry", says Norman Camerman.

"We look at the structures of AZT and other nucleoside analogs, compare these in three-dimensional space — architecturally, if you will — with each other and with

normal substrates, both in terms of their space-filling component and in terms of their distribution of what we call functional groups, and then postulate how these groups play a role in the compound's biological properties. And then we use this information to postulate guidelines that may be of use in the design and synthesis of new analogs".

"We've looked at some of the successful analogs, such as AZT and fluorodeoxythymidine, and also at unsuccessful analogs, such as cyanodeoxythymidine, and by seeing how they differ structurally, especially in the spatial and electronic configurations of their functional groups, we arrive at information on what parameters should be followed when designing analogs of this type".

" Both of us did equally well in all the subjects we ever took, and I guess when we advanced through high school and university it just naturally seemed that one could perhaps make more important contributions and do more important work if one went into science".

Norman does not look back on any unusual attraction to science from an early age either on his part or his brother's.

"Both of us did equally well in all the subjects we ever took, and I guess when we advanced through high school and university it just naturally seemed that one could perhaps make more important contributions and do more important work if one went into science. We tended to do things that were more chemical than physical, and so wound up going into chemistry. But even in chemistry we were more interested in chemistry that had biological ramifications".

At that time in graduate school, molecular structure was not being used much to understand the basis of drug action, so the Camermans started picking chemical projects with a biological interest, and they have continued ever since.

The application of their technique to AIDS research was "just a natural continuation of the work we were doing", says Norman.

"When AIDS came along, there was obviously a pressing need to develop anti-AIDS drugs. It seemed to us a natural thing to look at drugs that had shown some anti-HIV activity to see if we could come up with an explanation at the molecular level of how these drugs worked, and thereby perhaps formulate guidelines for the design of new and better agents".

Macrophages as HIV Infection Reservoirs

DR. MARK WAINBERG OF MONTREAL SUGGESTS HOW TO STIMULATE THEM SO DRUGS WILL GET RID OF HIV.

It is probable that before the appearance of the acquired immunodeficiency syndrome, most people without medical knowledge had never heard of T lymphocytes or T cells. Yet in the few years since the acronym AIDS came into common usage, all those with a serious interest in the malady must now be aware of the central role the T cells play in it.

The same general unawareness probably also applied to macrophages — scavenger cells that engulf invading disease organisms (antigens), and B cells — which make antibodies against them. Yet the interaction of these two types of white cell with HIV is still relatively little understood even by AIDS medical researchers. One of those trying to remedy this lack of knowledge is Dr. Mark A. Wainberg, a professor of microbiology and immunology at McGill University and researcher at the Lady Davis Memorial Institute for Medical Research, an affiliate of the Sir Mortimer B. Davis Jewish General Hospital in Montreal.

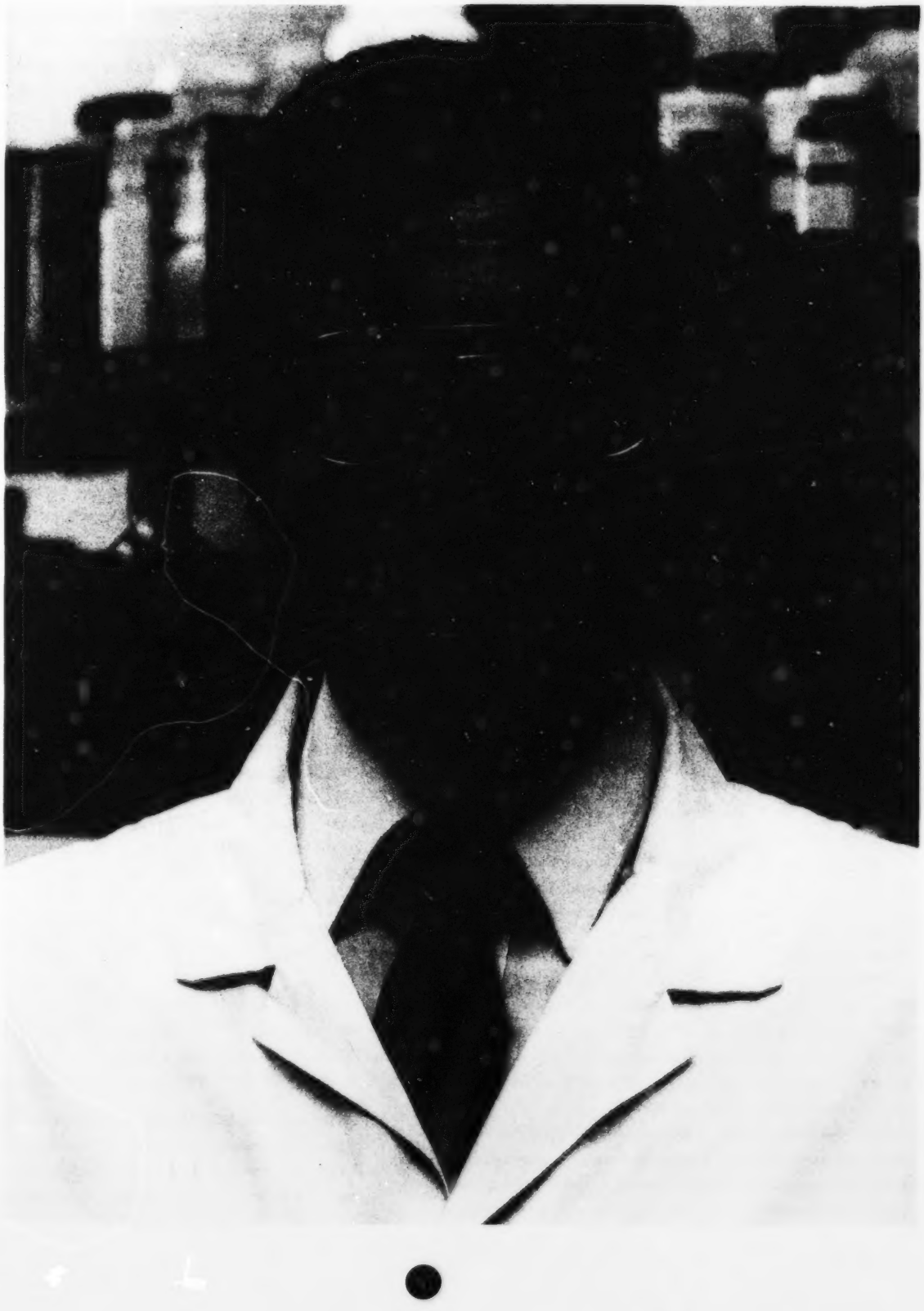
"In AIDS we all know that the major problem leading to disease is that the virus infects the T lymphocytes we call T helper cells through their receptor, which is called CD4, and those cells die", says Dr. Wainberg. "Well, those are not the only cells in the body that get infected. This problem, therefore, is not unique to T cells. And although we call the disease acquired immunodeficiency syndrome, its manifestations include far more than just derangement of the immune system".

"There's no question that macrophages are extremely important target cells for HIV-1[3]. There is a lot of evidence now, in fact, that macrophages are among the first cells to become infected. And they play an important role with regard to the spread of HIV-1 through the body to other cell types — including T cells — because they circulate and come into contact with them".

Because they are scavengers, macrophages attach very efficiently to the virus, says Dr. Wainberg. But unlike T cells, which die off quickly after HIV infection, macrophages remain in the body for a long time and continue to spread virus through it. There also is evidence that macrophages can cross the blood-brain barrier, thus infecting brain cells and producing neurological symptoms.

" It's absolutely crucial that this area of study become a priority for AIDS researchers, because we must recognize that the virus can replicate in many different cell types..."

As for B cells, not all types can be infected by HIV: in tissue culture at least, the only ones that can are those previously infected by another organism called Epstein-Barr virus (EBV). EBV causes infectious mononucleosis. It also transforms the B cells it infects, "immortalizing" them and causing them to persist in the body throughout the lifetime of the person who carries them. Such infections are present in a large majority of people,



who may, however, never show infectious mononucleosis symptoms.

"These infections don't affect our life expectancy", says Wainberg, "but the virus is within us nonetheless, and is constantly being replicated and constantly transforming B cells. These events are held in check, under normal circumstances, by our body's immune system: as quickly as the B cells become transformed, our body knocks them off. However, in AIDS patients, the immune mechanism may not function well.

"The transformed B cells can be infected by HIV-1 very easily", continues Wainberg, "and large quantities of the virus can be replicated within them. So it's possible these B cells may also play a role as a reservoir for the dissemination of HIV-1 through the body".

Wainberg and his colleagues wanted to know whether anti-viral drugs like AZT (azidothymidine, now called zidovudine) could inhibit viral replication in macrophages and B cells. For while it was known for some time that AZT could do so in T cells, "no one had ever asked these questions with regard to macrophages and B cells until we did", Wainberg says.

In a paper published in July 1989, Wainberg and Ph.D. student Michel Tremblay showed that AZT and similar drugs do in fact have the ability to inhibit HIV replication in B cells. But in another paper, Wainberg and François Boulerice (also a graduate student) show that AZT is completely ineffective for such inhibition in macrophages infected by the virus.

"This means we don't have effective drugs with which to treat viral infections in a cell like the macrophage", says Wainberg. "It's absolutely crucial that this area of study become a priority for AIDS researchers, because we must recognize that the virus can replicate in many different cell types, and devise our therapeutic strategies to confront it in all of them — not just in T lymphocytes".

One possible way of doing this is to take advantage of what is already known about gene expression in macrophages and T cells. One of the reasons macrophages do not inhibit HIV-1 replication is because they make low levels of an enzyme — thymidine kinase — necessary for AZT functioning. This enzyme phosphorylates AZT, converting it from an inactive to an active form.

"If we could figure out ways to get macrophages to make more of this enzyme on a regular basis, by treating cells in different ways, we might achieve a much higher level of success when we use this kind of drug", Wainberg says. "There are good reasons for making this hypothesis, because other groups at the U.S. National Institutes of Health have published that macrophages, when stimulated in certain ways, will in fact produce more thymidine kinase and become more susceptible to the anti-viral effects of AZT. We'd like to follow up on this".

Like so many of his Canadian colleagues, Dr. Wainberg's entry into AIDS-related research followed naturally from what he had been working on before AIDS appeared on the scene.

"I had been principally occupied as a medical researcher during the pre-AIDS period with studies that related to the ability of retroviruses to induce tumors in animals. What particularly interested me was how the body's immune system tried to defend it against those tumors. Most of my work was carried out on chickens infected with Rous sarcoma virus (the second virus discovered, in 1911, to cause animal tumors. The first was also a chicken virus called avian leukosis virus). One of the things we noticed about animal tumors caused by retroviruses was that they suppressed the animals' immune responses".

Wanting to know more about the molecular aspects of retroviruses, Wainberg took a sabbatical during the 1980 academic year in Dr. Robert Gallo's laboratory in the U.S. National Institutes of Health[4]. About a year after returning to his laboratory in Canada to resume work on how retroviruses could suppress the immune response, "we started hearing reports of this strange immune deficiency disease with all the trappings of a viral illness".

When Wainberg heard that there was strong evidence linking what was coming to be called AIDS to a virus, he said to himself: "There's a disease that corresponds exactly to the area I've been involved with for so many years. And so we decided to give it virtually our full attention".

Wainberg's laboratory thus became the first in Canada to start growing HIV and carrying out experiments on it. Their first publications on this work began emerging in 1985.

"We were fortunate in having access from the start to a P-3 bio-containment laboratory that the federal government funded", he says.[5] Such high levels of containment are essential for work with dangerous microorganisms.

A native of Montreal, Wainberg obtained his bachelor's degree in science from McGill University and his Ph.D. in microbiology and immunology from Columbia University. Most of his Ph.D. work related to the

influenza virus family. After receiving his doctorate in 1972, he spent two years as a post-doctoral student at the Hebrew University in Jerusalem, Israel, where he worked on cellular immunology. Since 1974 he has been at the Lady Davis Memorial Institute in Montreal and McGill University.

Wainberg first wanted to be a physician, but, as time passed, he realized he was more interested in the scientific than in the therapeutic aspects of medicine.

"Sometimes I regret the decision", he says. "If one is working in a clinically relevant area of investigative research there are advantages in having an M.D. after one's name."

Nonetheless, Wainberg says he is happy and honoured to be involved in AIDS research and believes it will make a real difference in the way in which the disease affects mankind.

"I think we need desperately to bring more good people into AIDS research so that, as a nation, we will generate the critical mass of individuals that can make a difference. I don't think we have succeeded as yet in attracting enough good, talented people to this field".

Why not?

"I think some people are afraid of coming into contact with the virus. People have all kinds of emotional reactions to the AIDS virus, and that's an understandable, if not a logical, fear. A second reason is that many of our good scientists are already committed to other problems, and they say 'these problems are important. We don't want to switch from working on a cure for cancer, or muscular dystrophy or multiple sclerosis to working on a cure for AIDS when we think what we're doing already is pretty important'. And I think that's valid".

Yet another factor keeping some researchers away from AIDS studies was that they thought a cure would soon be found, says Wainberg, and they were unwilling to make a commitment to a problem they did not think would be around for a long time.

"I think there's widespread recognition now that AIDS will be with us for the foreseeable future. And AIDS will not be the last important disease caused by a human retrovirus. I think we have just embarked on the era of human retrovirology, and with time we're going to find other retroviruses that cause other important diseases. There are already groups of scientists looking for linkages between some of the known retroviruses and diseases such as multiple sclerosis, for example, about which we don't have good understanding".

What Canada therefore needs to do, in Dr. Wainberg's opinion, is to encourage those starting out in medical research to regard AIDS as a priority.

[3] Two human immunodeficiency viruses have been identified: HIV-1 and HIV-2. The former is the cause of most cases in North America; Europe; East, Central and Southern Africa; and the Caribbean. HIV-2 appears most commonly in West Africa, but has also been found elsewhere.

[4] Dr. Gallo is known as the discoverer in 1980 of the first human retrovirus, human T-lymphotropic virus type I, or HTLV-I, and as the co-discoverer in 1983 of the AIDS virus, which he called HTLV-III. French scientists Françoise Barre-Sinoussi, Jean-Claude Chermann and Luc Montagnier in the same year discovered HIV, which they called lymphadenopathy-associated virus, or LAV. The name human immunodeficiency virus, or HIV, was agreed upon by an International Committee on the Taxonomy of Viruses in 1986.

[5] Through the National Health Research and Development Program (NHRDP).

Dr. Rafick-Pierre Sekaly's work in Montreal is designed to block HIV attachment to body cells without interfering with immune reactions.

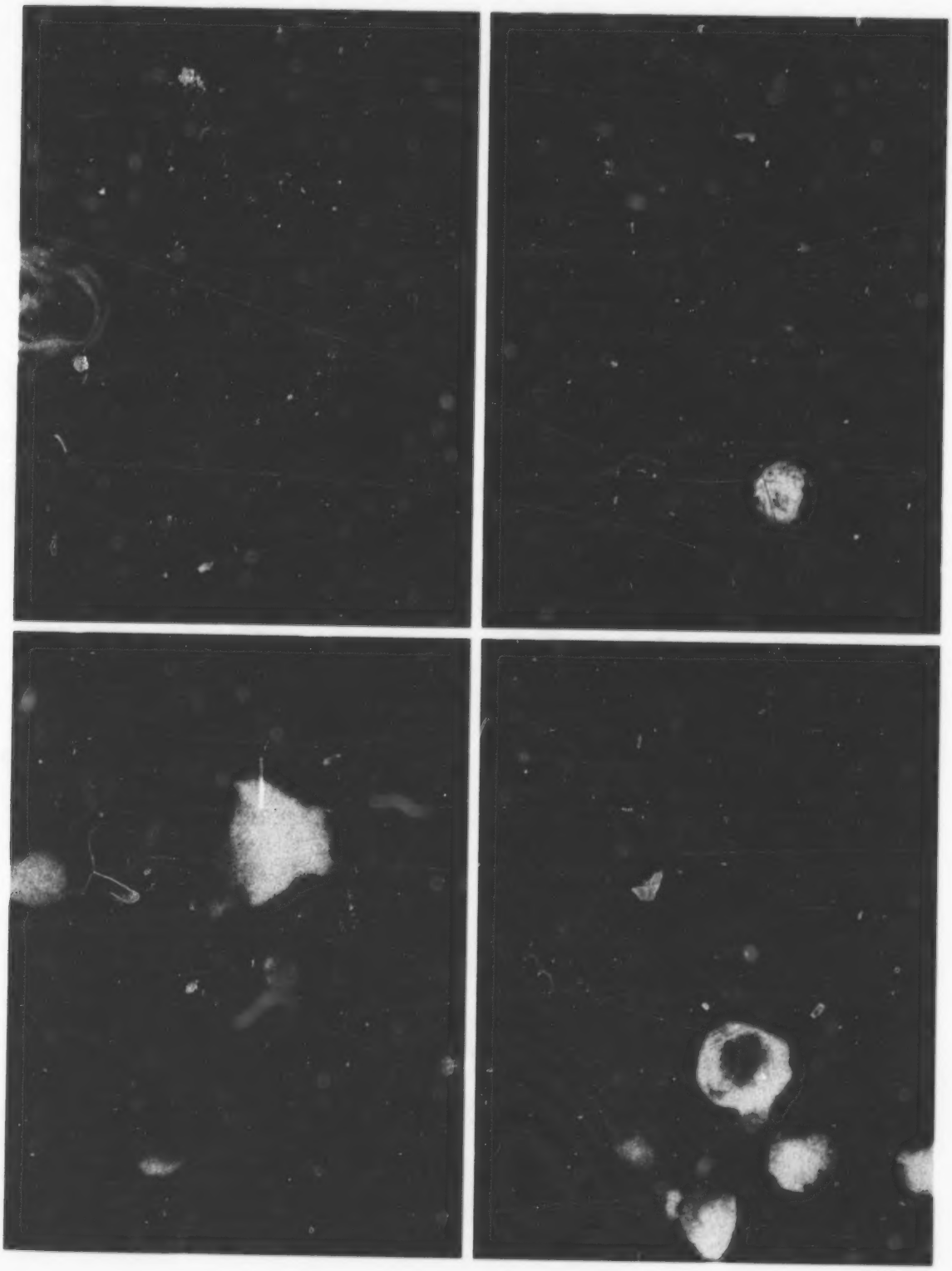
" I made the choice after I got through medicine.

Building Better Blockers

One of the ways in which medical scientists are trying to prevent the progression of HIV infection in people who have tested HIV-positive is to attempt to prevent the binding of the virus to their T cells.

I realized I wasn't a doctor, I was a researcher. In the medical profession there is much less incentive for innovation. It's very important for me to go where my curiosity leads me: it's my temperament. "

The site on the T cells to which the virus binds is the CD4 molecule (it does this by means of a protein on the virus envelope called gp120). The strategy is to try to intercept free, circulating viruses with "decoy" CD4s made in the laboratory with the help of what is called recombinant DNA technology. Once bound to the soluble, circulating CD4 molecules, these particular viruses cannot go on to infect the binding sites on the T cells. The hoped-for effect of the strategy is to saturate the binding sites on free HIV, thus decreasing the number of virus particles able to infect CD4-bearing cells.



A complicating factor is that CD4 is also thought to interact with other molecules produced by the body called Class II major histocompatibility molecules (MHCs). MHCs play a key part in the body's normal immune reactions, mobilizing large numbers of T cells, which as we saw are the "generals" of the body's immunological forces. If laboratory-made CD4 molecules were to be introduced into the body to inhibit the binding of HIV to CD4 molecules, it would not do for this therapeutic intervention to interfere with the body's normal immunological functions. One result might be that such large numbers of T cells would be mobilized by soluble CD4s that an insufficient number would be available to fight other infections that might happen along.

Dr. Rafick-Pierre Sekaly, of the Clinical Research Institute of Montreal, and his colleagues undertook to identify which regions of the CD4 molecule are important in MHC binding, and to determine whether these are the same regions to which gp120 binds — or whether indeed there is any relationship between the two. They found that, while there may be some overlap between the binding sites, the two are in fact distinct. This means that it should be possible to design CD4 molecules in the laboratory that can block HIV infection yet at the same time will not affect the body's normal immune response.

"Our experimental results give us great hope that we can design very specific inhibitors of the interaction of CD4 with gp120, but not of normal immune function", he says.

"What we are trying to do now, in collaboration with Genentech (a U.S. firm) is to make much smaller versions of these soluble forms of CD4, comprising only the sequences of CD4 implicated in binding to HIV, and to see whether they will in fact inhibit this binding. These are peptides — small molecules comprised of 10 to 15 amino acids".

Dr. Sekaly's group worked on these experiments in the laboratory for a year and a half. The scientists now are trying to see if what happens *in vitro* will also occur *in vivo*: they have begun to use a "transgenic" mouse, or one into whose genes a human gene has been incorporated. They expect to get some results within a few months. It is a highly competitive field. There are at least two or three groups in the U.S.A. and one in Europe working in the same area.

In addition to the implications Sekaly's work has for therapy for those already infected with HIV, there are others for vaccines designed to prevent such infections from occurring in the first place. Vaccines must be designed so as not to produce detrimental effects on the immune systems of those to whom they are administered. If, as Sekaly's work has shown, gp120 can mimic the normal immunological response of the body, when used in a vaccine it might set off all sorts of immunological false alarms, thus lessening the body's ability to react to normal infections.

"Gp120 is being used now in vaccine trials", says Sekaly. "I would fear that it could have deleterious effects on the system. The idea would be to make the pieces of gp120 as small as possible so that they do not elicit undesirable kinds of immune response".

" I decided it was not medicine I was after, it was really science, so I switched to science. I did my master's in immunology here in Montreal, and then I did a Ph.D. in Lausanne, Switzerland and a post-doctoral fellowship at the U.S. National Institutes of Health ".

Dr. Sekaly's entry into AIDS research stemmed from previous work he had done with the reactions between MHC and CD4 molecules. Because CD4 is also the receptor for HIV, it was a natural step.

"It became very obvious that all the work I was going to do on CD4/MHC was also going to involve work with HIV", he says.

Sekaly didn't set out originally to become a medical researcher: he was going to be a medical practitioner.

"I decided it was not medicine that I was after, it was really science, so I switched to science. I did my master's in immunology here in Montreal, and then I did a Ph.D. in Lausanne, Switzerland and a post-doctoral fellowship at the U.S. National Institutes of Health".

His basic motivations were curiosity and creativity.

"I made the choice after I got through medicine. I realized that I was not a doctor, I was a researcher. In the medical profession there is much less incentive for innovation. It's very important for me to go where my curiosity leads me: it's my temperament".

DR. C.Y. KANG OF OTTAWA USES INSECT CELLS TO PRODUCE THE LARGE QUANTITIES OF ANTIGENS NEEDED FOR AN HIV VACCINE.

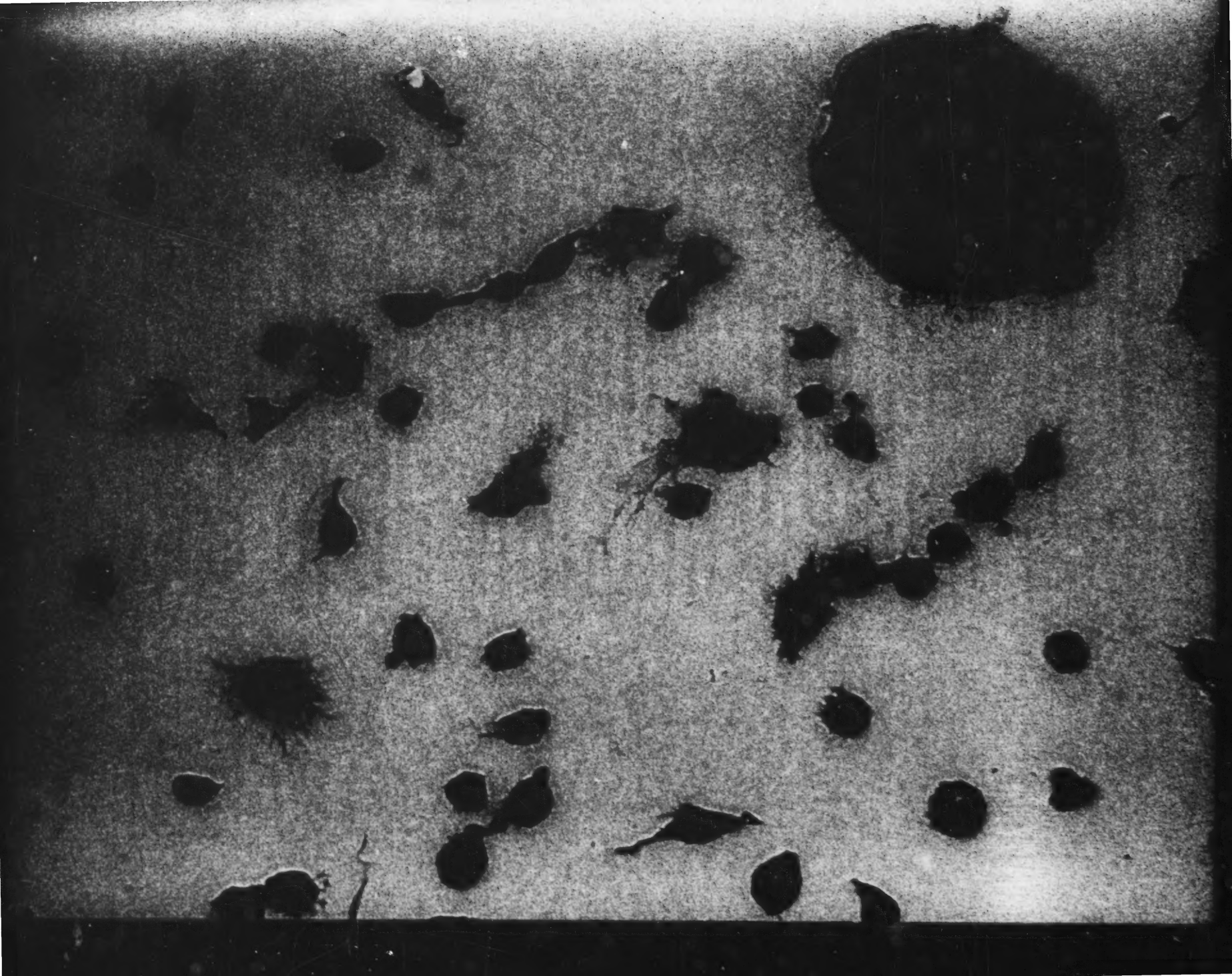
We have vaccines to protect us against poliomyelitis, measles, influenza and many other diseases caused by viruses. Why does it seem so difficult to develop a vaccine to protect us against HIV, the AIDS virus?

"... In fact I was very reluctant to get into HIV work because I didn't want to get on the bandwagon everybody else was riding. But as a retrovirologist, I felt I was obliged to get into AIDS research ".

The reasons are many. Most involve the highly unusual nature of the human immunodeficiency virus. Before we consider some of these reasons, let's review how a vaccine works.

A vaccine stimulates the body's immune system to react against a foreign invader such as a virus. It does this by introducing into the body some foreign substance that is recognized by the immune system as the disease agent, but is actually harmless. In a polio vaccine, for example, this is either a killed virus or an attenuated, live virus (i.e., one that has been rendered harmless).

Caterpillars as Antigen Factories



An attenuated live virus cannot safely be used as an HIV vaccine, however. Nobody would want to volunteer to be a test subject for a vaccine containing an attenuated live human immunodeficiency virus because of the risk that the attenuation would not be adequate and that the vaccine itself would produce an infection.

As for a killed vaccine, huge amounts of HIV would be necessary to produce it — posing unacceptable risks for those involved in its preparation. Furthermore, a killed HIV vaccine would not contain all of the virus proteins needed to make it work.

Fortunately, modern genetic engineering techniques make it possible to overcome these problems. They allow isolation of the HIV genes that direct the production of its proteins, which can then be produced in quantity in the laboratory. These proteins can be introduced into the body in the form of a vaccine to produce the desired immune response, and because they are only parts of the virus, they are not in themselves infectious.

Nevertheless, serious difficulties stand in the way of producing an effective yet harmless HIV vaccine. They include the following:

- ***Some HIV genes can mutate quickly and repeatedly. It is difficult for antibodies made by the immune system in response to one strain to recognize other, mutated strains.***
- ***Further research is needed to find a suitable animal model for AIDS, so the efficacy of the vaccine cannot be easily evaluated.***
- ***Nobody knows how long HIV's latent period may last. This makes it difficult for researchers to know how long they must wait before they determine that a trial vaccine has been successful.***

Despite these difficulties, many researchers continue to try to develop a vaccine against HIV. More than 20 such projects are underway around the world. A promising one is being pursued by a Canadian team under Dr.C.-Yong Kang at the University of Ottawa's Faculty of Medicine.

The idea behind HIV vaccines is to introduce parts of the virus (viral proteins) into the body to induce it to react as it would against the virus itself: that is, to produce antibodies against the proteins. Two candidate vaccines already licensed for human trial by the U.S. Food and Drug Administration contain an HIV surface glycoprotein (an antigen) to trigger production by the body of antibodies to neutralize the virus. However, it is not clear if such a vaccine will work, because some apparently

healthy yet HIV-infected individuals who already have adequate levels of antibodies against surface proteins still develop AIDS.

Dr. Kang's approach is to produce a vaccine containing not only proteins from the virus surface, but also those from its interior (or core), which are involved in its replication and assembly, and in addition, others that regulate its production. Such an approach is expected to provide further lines of defence beyond those assumed to result from the use of a vaccine containing only surface proteins. Although it is not known exactly how such protection might occur, it *is* known that core proteins of other viruses provide protection, for example in measles, human hepatitis B and influenza.

The surface, core and regulatory proteins for Kang's vaccine are produced by molecular engineering techniques in a novel system employing a virus that infects insects, known as a baculovirus.

In this system, a specific gene (the polyhedrin gene) of the baculovirus is replaced with the genes from HIV that direct the production of the desired proteins. The altered baculovirus is then inoculated into the cells of caterpillars. As a result, the caterpillar cells become, in effect, miniature protein factories, manufacturing large quantities of the proteins required for the vaccine. By replacing the usual high-cost fermentation facilities as vessels for virus culture, the caterpillar cells provide great savings in production costs. The caterpillar antigen factory system was developed in collaboration with the Institute of Virology in Oxford, England.

Initially, the major problem was that, "if you put two different genes into the baculovirus system, sometimes one would produce a very high level of proteins, but another would produce very little", says Dr. Kang. "And in protein engineering in biotechnology, the bottom line is to make large amounts of desirable proteins". However, his team found a solution to high level production early in 1989. Now, although this baculovirus system is based on a technique developed in the United States, the refinements achieved at the University of Ottawa make it unique.

What Kang and his group plan to do is to put together a number of HIV proteins in a vaccine, enabling them to combine to produce a four-tiered reaction against HIV. Kang speaks of the four tiers as "dams", which prevent virus/host cell interaction in different ways.

The first dam is designed to prevent the virus attaching to the host cell: if the virus cannot bind to the cell, it cannot enter it, and if it cannot enter it, it cannot infect it. The vaccine thus would interfere with the binding of the virus (by means of its envelope protein gp120) to the CD4 molecule on the outside of T4 lymphocytes, monocytes or macrophages.

If the virus somehow escaped that first dam and managed to enter the cell — for example by transmission from cell to cell — it would face a second dam. In order to infect a cell, the virus gene must unite with the genes of the cell. But HIV is a retrovirus (a virus that passes genetic information to a cell it has infected "in reverse" when compared with other viruses): its genes are contained in RNA (ribonucleic acid), while human genes are contained in DNA (deoxyribonucleic acid). It therefore cannot pass its genetic information directly, but requires an intermediary. HIV contains an enzyme, known as reverse transcriptase, to solve this problem: it enables the virus to produce a DNA copy of its RNA. The vaccine would interfere with this copying process. The third dam would inhibit the regulating proteins necessary for virus replication. And the fourth would interfere with assembly of the virus.

Kang's vaccine has one great potential advantage over other candidate vaccines. Not only could it be used in the conventional way vaccines are used — to prevent people from becoming infected with HIV in the first place. It also might be used with individuals who have *already been infected* but who have not yet become ill. Such individuals, it is hoped, could remain symptom-free indefinitely because their immune systems, which are still functioning, would be stimulated by the vaccine to continue to produce antibodies. It also might be possible to *cure* HIV infection by eliminating all infected cells from the body: T cells have a limited life, and *new* infection would be prevented by the vaccine.

"If we are armed with a high level of circulating antibodies against all HIV proteins — and with cytotoxic T-lymphocytes — in those asymptomatic patients", says Kang, "they may not develop full-blown AIDS. We know there are thousands walking the streets with HIV infection, though some of them do not know it".

Once the vaccine is made, it will be used to see if it can stimulate antibodies in laboratory animals. Trials with human volunteers will follow.

As noted earlier, HIV has been found in two different strains in humans, known as HIV-1 and HIV-2. This, of course, has implications for any potential HIV vaccine.

"The efficacy of an HIV-1 vaccine is very difficult to test because there is no ideal animal model", says Kang. "And because AIDS can take such a long time to develop, vaccine tests may take 10 years or more". In the meantime, however, the researchers can see what the vaccine produces in the way of antibody response.

"We do have animal models for HIV-2", says Kang, "and we will use the same strategy: we are making the antigens, and when we have produced all the HIV-2 proteins we will immunize rhesus monkeys and challenge them with live HIV-2 or Simian Immunodeficiency Virus (SIV) to make sure the vaccine protects them. (HIV-2 is closely related to SIV; therefore vaccination against HIV-2 is expected to be effective against SIV). If that is established, then we can extrapolate the results to the HIV-1 vaccine".

So far, Kang's group has succeeded in producing all the HIV-1 antigens using the novel baculovirus system, and by September 1989 had produced the first HIV-2 antigen. Testing of the HIV-2 vaccine for immunological responses in monkeys could be completed within two years, although tests showing whether the vaccine will provide full protection against the disease would take longer.

Dr. Kang's background is in veterinary science, which he studied in Denmark after leaving his native Korea. He came to Canada in 1966 and began working in virology at the Connaught Medical Research Laboratories at the University of Toronto. "The period of the mid-60s was the dawn of molecular biology", he recalls. "When I saw the incredible potential of molecular biology applied to virology, I decided to get into it. We were the first generation of molecular virologists".

Kang entered a Ph.D. program in virology at McMaster University in 1968, under the supervision of Dr. Ludvik Prevec. At the time, McMaster was one of the few centres in Canada in that discipline. Kang obtained his Ph.D. in 1971, after publishing five major papers in his three years of study.

"In 1970, something really important happened — the discovery of the reverse transcriptase enzyme by Dr. Howard Temin, the retrovirologist", says Kang. "I asked him if I could come to study with him as a postdoc and he accepted me, so I got into retrovirology in Dr. Temin's laboratory in 1971".

Up to that time, says Kang, "accepted dogma" in biology was that information flow in living systems was unidirectional: from DNA to RNA to protein.

"The discovery of this enzyme broke the central dogma, showing that genetic information can be transferred from RNA to DNA — a reverse flow, which is why it's called reverse transcriptase. And that really revolutionized genetic recombination work.

"In fact there are two pillars supporting today's genetic engineering. One pillar is this discovery of reverse transcriptase. The other is the discovery of restriction enzymes, which can recognize specific sites in the DNA molecule and cut them. Without these two discoveries we would not have been able to manipulate genes. They are the two most important discoveries from the point of view of genetic engineering".

Having entered retrovirology, Kang found the transition to HIV (itself a retrovirus) relatively smooth. But, he says: "In fact I was very reluctant to get into HIV work because I didn't want to get on the bandwagon everybody else was riding. But as a retrovirologist, I felt I was obliged to get into AIDS research".

As a boy, Kang was always interested in science. He would find discarded radios and record players, take them apart and re-assemble them and make them work.

"I was always curious about the mechanics of things and how they work", he says. "When I was in grade 7, I inherited my father's watch. Knowing I was a very curious boy, my father opened the back of the watch, showed me what was inside, and then told me: 'This is what's inside. Look at it. Now that you have seen it, never open it again!'"

Yong Kang never did. But in later years he obviously hasn't been able to resist opening up the genomes of living organisms and trying to find out what makes them work.

" When I was in grade 7, I inherited my father's watch. Knowing I was a very curious boy, my father opened the back of the watch, showed me what was inside, and then told me: 'This is what's inside. Look at it. Now that you have seen it, never open it again!'"

Creating an HIV Vaccine

DRS. LUDVIK PREVEC, KENNETH ROSENTHAL AND FRANK GRAHAM OF HAMILTON USE A RELATIVELY HARMLESS ADENOVIRUS AS A VEHICLE TO GET AN IMMUNITY-PRODUCING HIV GENE INTO THE BODY CELL.

Seventy percent of the world's population has been infected by an organism called human adenovirus type 5. It causes relatively harmless upper respiratory infections with which we are all familiar.

Large numbers of clinical trials have been conducted with vaccines for Ad5, as it is called by researchers. Successful vaccines have in fact been made to protect millions of military personnel against the virus. For although the symptoms it causes are not serious, Ad5 spreads rapidly under barrack conditions and could potentially disable large numbers of troops simultaneously.

Because of its relatively innocuous nature and the extent of clinical experience with vaccines employing it, three McMaster University researchers decided some time ago to use Ad5 as the basis for designing a new rabies vaccine. Using a form of genetic engineering known as recombinant DNA technique, Drs. Frank L. Graham, Ludvik Prevec and Kenneth L. Rosenthal succeeded in developing a rabies vaccine for wildlife, which is now being tested in Ontario.

They did this by substituting certain genes from the rabies virus into the Ad5 virus and re-packaging it as a new viral entity, so that, when introduced in a vaccine into an animal's body, it produced an immune response against rabies. A similar approach was used to develop a herpes vaccine. They call these new viral entities "adenovectors".

Having found that the method succeeded with rabies and herpes viruses, the group wondered why it would not work also with HIV, and they are now working on the assumption that it will. If they prove to be right, the result may be a new vaccine that will protect against infection by the AIDS virus.

Dr. Graham is an expert in adenovirus research. Dr. Prevec, a molecular virologist, and Dr. Rosenthal, an immunologist, had worked together on the rabies vaccine. When they considered introducing HIV antigens into Ad5, they saw a number of optional candidates: the envelope proteins, for example, or the core proteins, specifically one known as *gag* (group specific antigen).

"We decided for a number of reasons to go after the *gag* protein", said Dr. Prevec, "and we have taken that gene and put it into adenovirus, and have been able to get expression of the protein in the adenovirus vector. And we've injected mice — and more recently monkeys — and have been able to raise antibodies in those animals against the *gag* protein".

"...What we need to do is get killer cells to recognize virus-infected cells, and then those cells would be destroyed..."

As an immunologist, Dr. Rosenthal's chief interest since he completed his Ph.D. has been how the immune system protects against viruses. Says Rosenthal: "When AIDS came along, one of the major questions was, 'What immune response, if any, is important in controlling this

virus (HIV)?', and we don't yet know the answer to that. Antibodies don't seem to play that role. Our hypothesis is that cytotoxic T cells or CD8 positive killer lymphocytes are very important in controlling HIV replication and in preventing the infection from progressing to AIDS".

The group's work with adenovectors has a dual purpose, says Rosenthal: the vectors can be used not only in vaccines, but also as a research tool, because they allow the introduction of a single gene into a cell.

"Our hope is to use these recombinant adenovectors as a means of studying, first of all, what parts of HIV are recognized by T cells, and whether or not adenovirus vaccines will produce a protective immune response in animals and man. We're excited because so far we've constructed some Ad5 recombinants that contain the whole *gag* region and some with just the p24 (inside coat protein). And when cells are infected with these they make the HIV protein. And as Dr. Prevec said, when injected into mice and monkeys, they raise antibodies".

Most other researchers working on vaccines are using HIV envelope genes, and preliminary data seem to indicate such vaccines are not very effective.

"Our argument is that antibodies probably don't do much, and that what we need to do is get killer cells to recognize virus-infected cells, and then those cells would be destroyed. We know from animal studies of lymphocytic choriomeningitis virus that killer cells are important in controlling that infection, as they are in herpes infections".

One novel aspect of the McMaster vaccine is that it involves an infectious process. The adenovector takes the HIV gene directly into the body cells, where it replicates. This is not the case with killed virus vaccines, which simply introduce soluble proteins into the body and raise antibodies. That seems to be an important difference, because in HIV infection what is called the cell-mediated immune response appears to be more important than the production of antibodies.

Another interesting aspect derives from the fact that the adenovaccines administered to the military are oral vaccines.

"This could be quite important", says Rosenthal. "The adeno recombinants may end up being used as an oral vaccine. If you introduce the vaccine orally into the body, you tend to promote a mucosal immune response — and AIDS is an infection that enters the body through the genital mucous membranes".

Although no good animal model for HIV infection and AIDS exists, the McMaster group hope that the simian immunodeficiency virus (SIV), which is similar to HIV, can be used in their adenovectors to produce a vaccine that will protect monkeys against SIV. If this turns out to be the case, the monkey vaccine will be able to serve as a model for an HIV vaccine in humans: it would be reasonable to suppose that if an SIV vaccine provides protection to monkeys, the analogous HIV product should provide protection to humans. In fact, because some cross-infectivity occurs between SIV and HIV, it is even possible that the *monkey* vaccine may provide protection to *humans* against *HIV*.

The monkey work is important, says Prevec, because "we really have to know if we can protect an animal in this way against an AIDS-like disease".

The fact that Ad5 infections are so prevalent in humans may prove a stumbling block to the development of an Ad5-based HIV vaccine, the researchers admit: most adults may already be immune to Ad5 (though babies may not, and Ad5 could work with them). But this is not known for certain, and Ad5 was chosen as a vector because it was feasible to use it that way, and because it had in fact already been used in the rabies vaccine, which works. So even if Ad5 does not work as an HIV vaccine, one of the other 30-odd varieties of adenovirus could be chosen for similar attempts.

The McMaster researchers collaborate with Connaught Medical Research Laboratories on this work, exchanging scientific information and using monkeys kept in Connaught's facilities. Without these facilities it would be impossible to do the monkey work because there are no other good facilities available in which to keep infected monkeys. More of these are sorely needed, they believe. Such research also needs a better supply of post-doctoral students and better salaries with which to attract them.

McMaster University is an appropriate locale for the kind of interdisciplinary studies involved in the Ad5 vaccine work, because faculty members from many departments there are accustomed to collaborating on research. Dr. Prevec himself is an example of cross-disciplinarity: his undergraduate work was in geophysics at the University of Toronto. He grew up in Kirkland Lake in northern Ontario, and, as a result, had an interest in mining. "I was interested in nature, but knew nothing about biology", he says.

"There was a time in the late '50s and early '60s when physicists were being attracted to biology, and biophysics was the big thing. A lot of the big biological breakthroughs were coming from X-ray diffraction. So I got into biophysics. I started graduate school at the department of medical biophysics at the University of Toronto. If you were to poll the biology department at McMaster today you'd discover that there are five of us with physics backgrounds".

Dr. Rosenthal's interest in science was well developed by the time he went to high school: he did well in science subjects, was named "science student of the week" and won biology awards.

"As do many people, I enjoyed nature", he says, which was perhaps not easy for a child who grew up in Chicago. "I can remember when I was little my father taking samples of pond water, and us looking at it under the microscope. It was my father who bought my first microscope.

"When I first went into university I was interested in veterinary medicine: through high school I had worked as a kennel cleaner. But once I went to university and took courses in microbiology I became more interested in what caused diseases than in treating Mrs. Goldbloom's poodle. I guess it's just curiosity".

Dr. Rosenthal thinks science today is not a particularly attractive career choice for students, because the financial support for science is "marginal". He points to the proportion of the gross national product spent on science in Canada compared to its competitors and finds it wanting. Nor does he see Canadian industry filling the gap, as the federal government had hoped. Dr. Prevec says that if industry could be persuaded to spend a little more money it would be bound to help everyone in this area.

"The researchers in health sciences face the same problem as those in the biological sciences", says Prevec. "We all go for the same pot of money, and it's not that big. There are far more lucrative careers, and it doesn't take a smart student very long to discover that he could be working no harder in medical school and earning a lot more when he comes out at the other end".

Drs. Alan Bernstein and Sadhna Joshi of Toronto want to replace the HIV-susceptible stem cells in AIDS patients' bone marrow with transplanted HIV-resistant ones.

Transplanting HIV Immunity

When Sadhna Joshi was a little girl growing up in India, her mother wanted her to become a scientist, but she had a neighbour and best friend who wanted to become a heart specialist.

"She was 16 when I was five, so I also wanted to be a heart specialist", she laughs.

" ... Now I have changed my opinion about doctors versus scientists, and I think I am happier being a scientist because I am developing new ideas. If I had been a medical doctor not doing research, I might just be applying the techniques developed by other scientists ".

But Joshi went to France for high school and university and decided that she didn't want to get into the medical system.

"My French wasn't really good enough to pass on to the competitions for places in medical school, and in France if you don't get into medical school you don't get credit towards a B.Sc. for your first two years. My mother didn't want me to waste time, so I decided to go for a master's. In France, if you wish, you can do two doctoral degrees, so I did two of them — in molecular biology and biochemistry".

Today, Dr. Joshi is an associate professor and director of the new HIV laboratory of the department of microbiology in the University of Toronto's faculty of medicine. And she feels it is just as well she didn't enter medical studies.

"This is what I really wanted in the beginning", she says now, referring to her AIDS research. "I feel so happy because I feel I have solved my medical problem as well. Now I have changed my opinion about doctors versus scientists, and I think I am happier being a scientist because I am developing new ideas. If I had been a medical doctor not doing research, I might just be applying the techniques developed by other scientists".

The AIDS project in which Dr. Joshi is engaged is a collaborative one between Allelix Inc., of Mississauga, Ontario, for which she used to work, herself, and Dr. Alan Bernstein and colleagues at the Mount Sinai Hospital Research Institute of Toronto. The idea is to use genetic engineering techniques to transfer resistance to HIV to human bone-marrow cells in the laboratory, and then to transplant these cells into an AIDS patient. The transplanted cells would confer HIV resistance on the patient.

"The cells infected by HIV in AIDS patients are T cell lymphocytes and macrophages", says Dr. Joshi. "Both of these are produced by bone-marrow stem cells. One could transform[6] the bone-marrow stem cells with a retroviral vector[7] expressing an anti-HIV gene. Then one could expect that upon transplantation of these transformed bone-marrow cells into the patient, the blood cells that would result would also express these anti-HIV molecules, and would be resistant to HIV if they encountered it".

Experimental evidence has been produced in the United States showing that a process like this can take place in blood cells of those infected with human T-lymphotropic virus type I (HTLV-I), which is similar to HIV. Such a gene works by reconstituting the body's entire blood-forming system.

About two years ago, Dr. Joshi was working on cell transformation processes with Allelix. Knowing of Dr. Bernstein's research at Mount Sinai, Allelix approached him to propose that the two groups collaborate.

"We wanted to get into AIDS research, and I thought it might be a very good idea to use this type of approach for therapy", says Dr. Joshi. Both Dr. Bernstein and Dr. Louis Siminovitch, director of the Mount Sinai Research Institute, liked the idea, so an application was made to the Medical Research Council of Canada for research funding.

The retroviral vectors are constructed by the researchers in a complex laboratory process involving portions of HIV RNA, a species of bacterium found in human and animal bowels called *E. coli*, and other cells called a "packaging line". The desired viral sequences (genes) are inserted in *E. coli*, which in turn are used to "transfect" the packaging cells. This results in virus vector particles which are employed to infect the target cells (human bone-marrow cells).

The team uses two target sites on the viral RNA for the transformation process. The first is adjacent to what is called the primer binding site, which is the starting point for the process of viral replication. The idea is to introduce anti-HIV genes to compete with viral RNA for access to this site. Once incorporated into the viral genome in place of the viral RNA, these anti-HIV genes prevent virus replication.

The other target site is the point at which a so-called "packaging signal" originates. The packaging signal — a new discovery described at the 1989 International AIDS Conference in Montreal — initiates the process that constructs the virus's protein coat around its RNA. Dr. Joshi is now experimenting to see if she can incorporate a phony packaging signal into the virus to take the place of the real one.

"We are trying to fool the cell into packaging cellular messenger RNA instead of viral messenger RNA", she says. If this could be made to happen, HIV would cease to reproduce.

With the use of these methods, the group must show that HIV resistance can be conferred on both macrophages and T cells, because the two types differ in their response to HIV infection. This is first done by growing the macrophages and T cells in laboratory cultures and introducing HIV into the cultures: if the macrophages and T cells survive, they have shown HIV resistance.

Obviously it would be impossible to test the HIV-resistance of these cells directly in a human subject by injecting him or her with HIV, so a special kind of nude (i.e., hairless) mouse is used instead. It acts as a sort of incubator for the macrophages and T cells. This strain of mouse — called SCID, XIC-nude — is highly immunodeficient, and thereby simulates a patient with AIDS.

Several months after introducing the transformed human macrophages and T cells into the mouse, the animal is "challenged" with an injection of HIV. If the animal survives, the experiment has proved that the macrophages and T cells are HIV-resistant.

The final step will be to transfer such HIV-resistant cells into volunteers with AIDS by surgical transplant, to see whether the HIV resistance continues to be conferred as the body's bone-marrow manufactures new macrophages and T cells. The researchers believe that AIDS can probably be reversed by such therapy. Brain damage resulting from HIV infection may not be completely curable by this therapy, Dr. Joshi says, but it will be useful for persons with AIDS without brain disorders — and it may even prove useful for the kind of brain damage resulting from abnormal production of growth factors by HIV-infected blood cells.

Dr. Joshi worked for more than eight years in France with plant and animal viruses, then came to Canada and Allelix. There she learned how to transfer antifungal genes from one plant to another, in an attempt to make tobacco plants resistant to fungus infections.

About this time she wanted to work on a virus project of her own, and her superiors at Allelix said, "What about AIDS?"

"So I said, 'Great — that's the best thing I could do'. I liked the plant virus work very much but I still felt that it was not that useful, so now I am extremely happy to be working on AIDS".

When a position became available at the University of Toronto, Dr. Joshi moved there. When this was written she occupied a temporary laboratory in the Banting Institute, waiting for her new HIV laboratory to be built.

Does she have any qualms about dealing with the virus that causes AIDS?

"No. You have to be careful. But you are not safe even on the streets", she replies. "At least here you are doing something useful".

One of the difficulties Dr. Joshi has found in her AIDS work is obtaining research materials in Canada, such as plasmids (small lengths of genetic material). She had to find researchers in the United States who were willing to supply them. What's needed, she thinks, is some sort of collaborative clearing house in Canada through which Canadian scientists working on HIV could pool their research materials.

[6] A cell can be "transformed" by introducing genes from another cell with different characteristics into its genetic material (DNA). The transformed cell will thereafter express the characteristics of the introduced genes.

[7] Retroviral vectors are, in effect, new viral varieties constructed in the laboratory from parts of existing viruses. They allow transfer of desired genetic characteristics into their target cells.

Canada and AIDS in Africa

HOW AN OUTBREAK OF CHANCROID IN WINNIPEG LED DRS. ALLAN RONALD AND FRANCIS PLUMMER TO ESTABLISH AN AFRICAN PROGRAM OF WORLD RENOWN.

In 1975 an outbreak of a venereal disease that was difficult to diagnose appeared in downtown Winnipeg. At first public health officials thought it was genital herpes, but because they were uncertain they called in a team from the U.S. Centers for Disease Control in Atlanta, Georgia. The Americans agreed that it looked like the textbook pictures of chancroid.

Chancroid is caused by an organism called *H. ducreyi*, a parasitic bacillus which produces sores on the labia and penis. Although rare in Canada in 1975, the disease is still common in areas of the Orient, Africa and the West Indies. Doubtless one reason it had been little studied here is that it was relatively harmless, causing no lasting effects as syphilis does, for example.

Some years earlier, Dr. Allan R. Ronald, now chief physician and head of the University of Manitoba department of internal medicine, had developed an infectious disease unit at the Winnipeg General Hospital. During the Winnipeg chancroid outbreak, he and one of his trainees, Dr. G. Hammond, began to study *H. ducreyi*, and the two became expert enough to publish several papers on it.

"Because of that", says Ronald, "the World Health Organization approached me in 1979 and said, 'There's a big problem in Kenya with genital ulcers and the microbiologist there has approached us for help. Could we put you in touch with him?'"

The Universities of Nairobi and Manitoba then approached the Canadian International Development Agency (CIDA), the International Development Research Centre (IDRC) and private groups for financial support for research. The result was the establishment in Nairobi of a clinic on sexually-transmitted diseases that is today regarded worldwide as a leader in African AIDS research and prevention.

The transition from the focus on chancroid to AIDS was fortuitous, and happened as follows. The story demonstrates the value of the Medical Research Council's policy of backing research whose end-result may not be obvious, as long as it is good science.

In 1981, Dr. Francis A. Plummer went from Canada to Nairobi for a year and shifted the emphasis of the program from genital ulcers to the problems of women and babies resulting from sexually-transmitted diseases. They were attending a clinic at the Pumwani Maternity Hospital. During this time the Institute of Tropical Medicine in Antwerp, Belgium, also became involved. The following year a clinic was set up for prostitutes, with the collaboration of Nairobi City Council.

" We were able to see a marked drop in the incidence of sexually-transmitted diseases because we treated the source of a significant number of these ".

"We were able to see a marked drop in the incidence of sexually-transmitted diseases because we treated the source of a significant number of these", says Ronald. "Since 1982 we have published more

than 100 papers."

As late as 1985 (when the University of Washington in Seattle also became a collaborating institution), there were still very few cases of AIDS in Kenya and no evidence the virus was spreading. But in that year the investigators did a retrospective epidemiological study on the blood samples of its patients that it had kept since the program's inception.

"To our horror, we found that the incidence of HIV-positives went from 4 per cent of prostitutes in 1981 to 65 per cent in 1985", says Ronald. "The results were published in 1986, and that's when we got into AIDS research. Now AIDS work constitutes about two thirds of our total".

When clinic doctors saw how rapidly HIV infection was spreading among Nairobi prostitutes, they hypothesized that chancroid lesions might be involved, and their studies showed this indeed to be the case.

"Suddenly, chancroid, from being a disease no one cared about, has become identified by WHO as being, after AIDS, almost the number two priority", says Ronald. "Chancroid is accounting for between one half and two thirds of the heterosexual transmission of HIV in some areas of Africa".

Adds Ronald: "Anything that breaks down the genital tract and causes lesions provides a portal of entry and of egress for the virus. The risk of a man getting infected with HIV from regular unprotected sex with an HIV-infected woman with a normal, healthy genital tract is probably between 1 per cent and 2 per cent a year. With an HIV-infected woman with a genital ulcer, the risk is probably 80 per cent.

"When a patient with HIV infection gets inflammation, the virus is recruited to the lesion. It makes perfect sense. We presented the first data on this, and now others have confirmed it. Everybody who has started looking has seen that if HIV-infected women start getting disease in their genital tract, the incidence of HIV infection in their sexual partners rises steeply. Perhaps even chlamydia* is an important risk factor for HIV infection".

Genital ulcers in men also increase their chances of becoming infected with HIV through a sexual partner. In a recent study, the Nairobi clinic showed that HIV-infected men were three times as likely to have had a recent history of genital ulcers. Uncircumcised men also are at much greater risk of HIV infection than men who are circumcised, for reasons not entirely clear. Preliminary studies have shown that the geographic distribution of male circumcision practices corresponds extremely well with the known patterns of HIV infection in Africa: in most areas with high HIV

Dr. Allan Ronald

" ... We didn't develop a plan until the Royal Society of Canada produced its AIDS study, and I think we could easily have had one two years earlier. People saw the problem back in 1985, and we should have done then what the Royal Society did in 1988 ".

prevalence, circumcision is not practised. In the African studies more than 95 per cent of attributable risk in men with sexually-transmitted disease was found to be due either to genital ulcers or to the presence of a foreskin.

The Medical Research Council of Canada began supporting the chancroid work in 1977, and as such was the only agency in the world funding research on *H. ducreyi* until 1988, Ronald says. The funding was provided simply because it was good science, and it has paid off by helping to make the Manitoba group the leading world centre in that disease. "If MRC hadn't funded us, this wouldn't have occurred", says Ronald.

Since 1984 Dr. Francis Plummer on an MRC scholarship has directed its research program. During the ten years since the program began five MRC research fellows have received research training in international health,

epidemiology, and infectious diseases in the Nairobi-Manitoba Program. An additional five Canadians and approximately 12 Kenyans have had research training in this program. Besides research, the clinic carries out a strong educational program amongst its clients, promoting practices — such as condom use — that reduce the risk of infection from HIV and other sexually-transmitted diseases. At first, says Ronald, condoms were laughed at by prostitutes, but their use has increased dramatically as the women have seen the spread of the HIV epidemic.

Another major focus of the clinic's studies has been to study HIV spread among heterosexual couples. Among many of these, HIV or other sexually-transmitted diseases are introduced by sexual contact with other partners. For those couples in which one partner has become HIV-infected, it appears that staying together and continuing to practise monogamy will limit HIV spread.

"Our concern is to assure people that their risks are small if they both have a healthy genital tract, and the best way to ensure this is to prevent sexually transmitted diseases," says Ronald.

Transient populations are another area of concern for the clinic personnel. In Africa, men commonly leave their families behind when they work in a distant community. The extra-marital sexual encounters this promotes increase the spread of sexually-transmitted diseases.

"This is potentially a time-bomb in terms of HIV", says Ronald.

In terms of Canada's own problems in dealing with the AIDS epidemic, Ronald identifies three areas. One was the relative weakness in retrovirology — and in virology as a whole — when the epidemic began, and also in the epidemiology of infectious diseases.

A second problem was that "we didn't develop a plan until the Royal Society of Canada produced its AIDS study, and I think we could easily have had one two years earlier. People saw the problem back in 1985, and we should have done then what the Royal Society did in 1988".

Furthermore, the Canadian government should be providing some kind of response to the Royal Society study, noting what it agrees with and what it doesn't, and holding itself accountable on at least a semi-annual basis, says Ronald.

A third problem is that Canada has not succeeded in getting enough good young Canadians into AIDS work, Ronald says. Part of the fault for this lies with the academic

community: the good researchers in the area should be producing clones of themselves. This could be done by attracting post-doctoral students into the field.

More professional strength is needed in clinical trials, Ronald says. And well-organized AIDS centres that work together are needed in major urban centres.

Ronald would like to see the Medical Research Council develop a strategic plan for AIDS research that would make use of the capabilities of all the other Canadian agencies involved. So far, the agency has not seen this as its role. Traditionally, MRC has been committed to supporting and encouraging excellence in science, and it has fulfilled this role well. However, Ronald thinks AIDS is a special case and demands special leadership.

" ...It was mostly British-style medical microbiology, with people based in the lab and seeing patients from that base. They didn't attract students into the discipline, so we still have a lack of personnel in the field 20 years later ".

After his graduation in medicine from the University of Manitoba, Ronald trained in Baltimore as an internist. He later went for a year to Pakistan with Dr. Theodore Woodward, "a grandfather figure in infectious diseases", and then, at Woodward's suggestion, to Seattle, where he specialized in Woodward's field. In 1968 he returned to the University of Manitoba.

"There was very little infectious disease as a clinical discipline at that time in Canada", Ronald recalls. "It was mostly British-style medical microbiology, with people based in the lab and seeing patients from that base. They didn't attract students into the discipline, so we still have a lack of personnel in the field 20 years later".

After returning to Winnipeg, Ronald started developing the infectious disease unit at the university, and by 1972 began getting fellows to train in the discipline.

"We've now trained 51. About half the people in infectious diseases in Canada have trained in Winnipeg."

Dr. Francis Plummer

[8] A special type of bacterium, one species of which, C. trachomatis, is a major cause of non-gonorrheal urethritis, and some other genital infections (among others).

Conquering AIDS

WHAT RESEARCH REMAINS TO BE DONE?

Reflecting upon everything this group of distinguished Canadian researchers told me, I could not help being struck by a number of similarities in their backgrounds, and a number of opinions they hold in common.

All, for example, became involved in AIDS research as a result of work they were previously doing, or interests they were already pursuing. Repeatedly I heard that the transition from their previous research to working on AIDS was a natural one. Almost all made that transition willingly — though one was initially reluctant to join what he saw as a 'bandwagon'. The move was made for a variety of reasons: the scientific challenge of AIDS research or its perceived social worth, or because the individuals felt unusually qualified to undertake it.

Once involved in AIDS research, all appeared happy to have made the move. They showed excitement over their achievements and the intellectual rewards of their work, which is now of course generally recognized as one of the major medical and social challenges of today. It is this challenge to their scientific ingenuity and skill that is clearly the major motivation of the group as a whole, with social or humanitarian usefulness running a close second.

" Initiatives should be taken to increase the number of people involved in AIDS research. The lack of human resources is and will remain a serious impediment to all AIDS-related activities in both research and health care ".

Many common elements can be found in the early backgrounds of these scientists. Curiosity obviously was a strong trait among them as children. Parental encouragement of their scientific tendencies was often a positive factor in their development. The scientist-to-be was apparent at an early age in one or two cases — though not all.

A number started out with the idea of becoming physicians or veterinarians, then realized their bent was scientific investigation rather than clinical care. Financial reward did not seem important as a motivation in any of the group: more than one noted that more money could be made in the private practice of medicine or in other professions.

The dominant attraction of medical research to its practitioners, if this group is at all typical, is the urge to know, the challenge to solve the biological puzzle, or the desire to find "a better way" to prevent, treat or cure disease, as one of them put it.

Those of them who, like our group, are good at their job, can think of none better: their working days are long, but they become completely absorbed in what they are doing, sometimes to the exclusion of most of the "normal" interests of other people.

Notably absent from their concerns was a fear of working with the human immunodeficiency virus — a fear that has shown itself to be so great among some Canadians that it has repeatedly led to illogical acts of social discrimination foreign to the underlying ethic of the country. Those who work with this dangerous virus take precautions. They use care in their work. But they show no evidence of fear of the organism they are constantly probing, disassembling and modifying. They are used to dealing with biological hazards. As one said: "You are not safe even on the streets".

Most of the researchers felt strongly that scientific concerns are not treated as seriously as they should be by Canadians. More than one felt that the educational system fails to prepare young people adequately for careers in science. A common thread in interview after interview was the belief that Canada, as a nation, does not devote enough money to science and technology as a proportion of its gross national product. Another repeated theme was the lack of sufficient funds specifically for medical research in Canada. None of these researchers complained for themselves; rather they spoke for funding of the activity as a whole.

Some spoke of specific needs: for closer contact between AIDS researchers across the country and even shared resources, and for central animal facilities.

But the most compelling need in AIDS research, it became apparent, is for more talented, committed investigators in the field. Though the researchers did not say so, what these interviews show is that the paramount requirement in Canadian medical research is for more people just like themselves.

Interestingly, some of the researchers' comments run parallel with some of recommendations of the Royal Society of Canada[9].

"Initiatives should be taken to increase the number of people involved in AIDS research", states the Society. "... the lack of human resources is and will remain a serious impediment to all AIDS-related activities in both research and health care".

It seems clear to many of those best qualified to judge that, despite the high quality of much of the research being carried out, Canada's scientific attack on the HIV/AIDS problem has not yet reached a level commensurate with its magnitude and urgency. And it is unlikely that the situation will change significantly until major initiatives are undertaken. What sort of initiatives?

Increasing funding is certainly one: as in other scientific areas in Canada, the lack of adequate funds is undermining the progress of medical research, and HIV and AIDS investigations are no exception. More funds are needed for support of projects like those described in this publication, for researchers' salaries when the usual sources (e.g., universities) cannot meet the need, and for training of new recruits to the field. The MRC alone could put to good use at least three times the amount (about $1.1 million) that it currently spends on HIV/AIDS research.

In addition, because Canada cannot find all the postdoctoral students it needs within this country, we must be able to attract well-qualified people from other countries. In the United States, where many of the best are to be found, we are simply unable to compete because of the low level of remuneration we currently provide.

I started this final chapter by noting some attitudes and opinions shared by the people I interviewed. One more should be mentioned: the hope — one might almost say expectation — that they will succeed in their endeavours.

This might be thought to go without saying, because without hope, one could hardly undertake a research project, let alone a career in medical research.

But it is nonetheless noteworthy, and it seems to be common among leaders in medical science. One of the greats — Dr. Lewis Thomas, President Emeritus of Memorial Sloan-Kettering Cancer Center in New York City — wrote in *The Youngest Science: Notes of Medicine-Watcher* in 1983:

"I look for the end of cancer before this century is over. It used to be the convention for people in my position to guess fifty years out, just to be safe, but I have become much more optimistic in the last few years. Indeed, I now believe it could begin to fall into place at almost any time, starting next year or even next week, depending on the intensity, quality, and luck of basic research. The world of medicine is becoming filled with the prospect of surprises, and this will surely be one of them".

Regarding the subject of this book, he wrote in *Scientific American* in 1988:

"In a long lifetime of looking at biomedical research, I have never seen anything to touch the progress that has already been made in laboratories working on the AIDS virus. Considering that the disease was recognized only seven years ago and that its agent, HIV, is one of the most complex and baffling organisms on earth, the achievement is an astonishment".

Scientific research represents a long-term investment in the health of the nation, and we must not expect immediate results. Nevertheless, experience has shown that success often appears with unexpected haste. In the case of HIV and AIDS, this must remain our fervent hope.

[9] *AIDS: A Perspective for Canadians*, Summary Report and Recommendations. Ottawa, Royal Society of Canada, 1988.